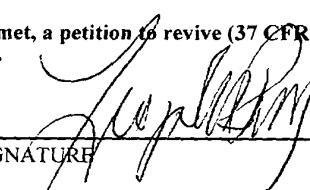
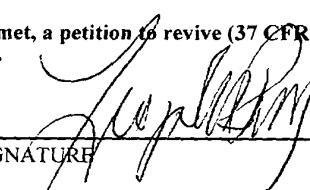
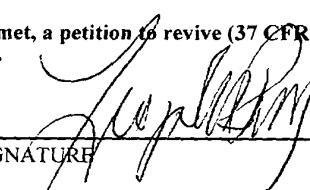


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|---|--|--|--|---|
| FORM PTO-1390 (Modified) (REV 10-95) | | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | | ATTORNEY'S DOCKET NUMBER 10441 |
| TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371 | | | | U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR 08/765588 |
| INTERNATIONAL APPLICATION NO PCT/AU96/00094 | INTERNATIONAL FILING DATE 22 February 1996(22.03.96) | PRIORITY DATE CLAIMED (02.03.95),(20.11.95), and(22.12.95) | | |
| TITLE OF INVENTION A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME | | | | |
| APPLICANT(S) FOR DO/EO/US Nicholas Kim Hayward, Gunther Weber, Sean Grimmond, Magnus Nordenskjold, and Catharina Larsson | | | | |
| Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: | | | | |
| <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). | | | | |
| Items 11 to 16 below concern document(s) or information included: <ol style="list-style-type: none"> <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input type="checkbox"/> A FIRST preliminary amendment. A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input checked="" type="checkbox"/> Other items or information: Courtesy Copy of international application | | | | |

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|---|---|---|---------------------|--|--|---|
| U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR | INTERNATIONAL APPLICATION NO. PCT/AU96/00094 | ATTORNEY'S DOCKET NUMBER 10441 | | | | |
| 17. The following fees are submitted: | | CALCULATIONS PTO USE ONLY | | | | |
| BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : | | | | | | |
| Search Report has been prepared by the EPO or JPO | | \$910.00 | | | | |
| International preliminary examination fee paid to USPTO (37 CFR 1.482) | | \$700.00 | | | | |
| No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) | | \$770.00 | | | | |
| Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO | | \$1,040.00 | | | | |
| International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... | | \$96.00 | | | | |
| | | \$1,040.00 | | | | |
| ENTER APPROPRIATE BASIC FEE AMOUNT = | | | | | | |
| Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). | | <input type="checkbox"/> 20 <input type="checkbox"/> 30 \$0.00 | | | | |
| CLAIMS | | NUMBER FILED | NUMBER EXTRA | RATE | | |
| Total claims | 52 | - 20 = | 32 | x \$22.00 \$704.00 | | |
| Independent claims | 10 | - 3 = | 7 | x \$80.00 \$560.00 | | |
| Multiple Dependent Claims (check if applicable). | | | | <input type="checkbox"/> \$0.00 | | |
| | | TOTAL OF ABOVE CALCULATIONS | | = \$2,304.00 | | |
| Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). | | | | <input type="checkbox"/> \$0.00 | | |
| | | SUBTOTAL | | = \$2,304.00 | | |
| Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). | | | | <input type="checkbox"/> 20 <input type="checkbox"/> 30 + \$0.00 | | |
| | | TOTAL NATIONAL FEE | | = \$2,304.00 | | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). | | | | <input type="checkbox"/> \$0.00 | | |
| | | TOTAL FEES ENCLOSED | | = \$2,304.00 | | |
| | | | | Amount to be: <input type="checkbox"/> \$ | | |
| | | | | refunded <input type="checkbox"/> \$ | | |
| | | | | charged <input type="checkbox"/> \$ | | |
| <p><input checked="" type="checkbox"/> A check in the amount of \$2,304.00 to cover the above fees is enclosed.</p> <p><input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p><input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 19-1013 A duplicate copy of this sheet is enclosed.</p> | | | | | | |
| <p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p> <p>SEND ALL CORRESPONDENCE TO:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="padding: 10px; vertical-align: top;"> Leopold Presser Registration No.: 19,827 Scully, Scott, Murphy & Presser 400 Garden City Plaza Garden City, NY 11530 (516)742-4343 </td> <td style="width: 33%; vertical-align: top; padding: 10px;"> SIGNATURE  Leopold Presser NAME 19,827 REGISTRATION NUMBER December 17, 1996 DATE </td> </tr> </table> | | | | | Leopold Presser Registration No.: 19,827 Scully, Scott, Murphy & Presser 400 Garden City Plaza Garden City, NY 11530 (516)742-4343 | SIGNATURE  Leopold Presser NAME 19,827 REGISTRATION NUMBER December 17, 1996 DATE |
| Leopold Presser Registration No.: 19,827 Scully, Scott, Murphy & Presser 400 Garden City Plaza Garden City, NY 11530 (516)742-4343 | SIGNATURE  Leopold Presser NAME 19,827 REGISTRATION NUMBER December 17, 1996 DATE | | | | | |

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)

Applicant(s): Nicholas Kim Hayward, et al.

Docket No.

10441

Serial No.
unassignedFiling Date
HerewithExaminer
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N/A

Invention. A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

I hereby certify that this New PCT Application

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is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under
37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231 on
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08/765588
89 Rec'd PCT/PTO 17 DEC 1996

- 1 -

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE
ENCODING SAME

5 The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present
10 invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOS.) for
15 the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to
20 imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Vascular endothelial growth factor (hereinafter referred to as "VEGF"), also known as vasoactive permeability factor, is a secreted, covalently linked homodimeric glycoprotein
25 that specifically activates endothelial tissues (Senger *et al.*, 1993). A range of functions have been attributed to VEGF such as its involvement in normal angiogenesis including formation of the corpus luteum (Yan *et al.*, 1993) and placental development (Sharkey *et al.*, 1993), regulation of vascular permeability (Senger *et al.*, 1993), inflammatory angiogenesis (Sunderkotter *et al.*, 1994) and autotransplantation (Dissen *et al.*, 1994) and
30 human diseases such as tumour promoting angiogenesis (Folkman & Shing, 1992), rheumatoid arthritis (Koch *et al.*, 1994) and diabetes related retinopathy (Folkman & Shing, 1992).

- 2 -

VEGF is, therefore, an important molecule making it a potentially valuable target for research into therapeutics, prophylactics and diagnostic agents based on VEGF or its activities. There is also a need to identify homologues or otherwise related molecules for use as an alternative to VEGF or in conjunction with VEGF.

5

In work leading up to the present invention, the inventors sought the multiple endocrine neoplasia type I susceptibility gene (MEN1). Surprisingly, the inventors discovered that a genetic sequence excluded as a candidate for the MEN1 gene was nevertheless a new growth factor having some similarity to VEGF. Furthermore, the growth factor of the

10 present invention is an effector molecule for primary and central neurons.

Accordingly, one aspect of the present invention comprises a biologically isolated proteinaceous molecule comprising a sequence of amino acids which:

(i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID

15 NO:2; and

(ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

Another aspect of the present invention provides a biologically isolated proteinaceous molecule having the following characteristics:

20 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;

(ii) exhibits at least one property in common with VEGF.

25 A related aspect of the present invention contemplates a biologically isolated proteinaceous molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

30 (ii) exhibits at least one of the following properties:

(a) ability to induce proliferation of vascular endothelial cells;

(b) ability to interact with *flt-1/flk-1* family of receptors;

- 3 -

- (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

By "biologically isolated" is meant that the molecule has undergone at least one step of purification from a biological source. Preferably, the molecule is also biologically pure meaning that a composition comprises at least about 20%, more preferably at least about 40%, still more preferably at least about 65%, even still more preferably at least about 80-90% or greater of the molecule as determined by weight, activity or other convenient means, relative to other compounds in the composition. Most preferably, the molecule is sequencably pure.

Another preferred aspect of the present invention provides the molecule in recombinant form.

According to this aspect of the present invention, there is provided a recombinant molecule comprising a sequence of amino acids which:

- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and
- (ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

A related aspect of the present invention is directed to a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with VEGF.

A further related aspect of the present invention contemplates a recombinant molecule having the following characteristics:

- 30 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

- 4 -

- (ii) exhibits at least one of the following properties:
- (a) ability to induce proliferation of vascular endothelial cells;
 - (b) ability to interact with *flt-1/flk-1* family of receptors;
 - (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

The present invention also contemplates genomic or partial genome clones encoding a proteinaceous molecule having at least about 15% amino acid similarity but at least about 5% dissimilarity to SEQ ID NO:1.

10

- The amino acid sequence set forth in SEQ ID NO:2 corresponds to human VEGF (referred to herein as "VEGF₁₆₅"). Accordingly, the molecule of the present invention is VEGF-like or is a homologue of VEGF but comprises an amino acid sequence which is similar but non-identical to the amino sequence of VEGF. Although the present invention is exemplified using a human VEGF-like molecule, this is done with the understanding that the instant invention contemplates the homologous molecule and encoding sequence from other mammals such as livestock animals (e.g. sheep, pigs, horses and cows), companion animals (e.g. dogs and cats) and laboratory test animals (e.g. mice, rats, rabbits and guinea pigs) as well as non-mammals such as birds (e.g. 15 poultry birds), fish and reptiles. In a most preferred embodiment, the VEGF-like molecule is of human origin and encoded by a gene located at chromosome 11q13. The present invention extends, therefore, to the genomic sequence or part thereof encoding the subject VEGF-like molecule.
- 20
- 25 Preferably, the percentage similarity is at least about 30%, more preferably at least about 40%, still more preferably at least about 50%, still even more preferably at least about 60-70%, yet even more preferably at least about 80-95% to all or part of the amino acid sequence set forth in SEQ ID NO:2.

- 30 In a particularly preferred embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:4 or is a part, fragment, derivative or analogue thereof. Particularly preferred similarities include about 19-20%,

- 5 -

and 29-30%. Reference herein to derivatives also includes splice variants. Accordingly, the present invention extends to splice variants of SOM175. Examples of splice variants contemplated by the present invention include but are not limited to variants with an amino acid sequence substantially as set forth in at least one of SEQ ID NO:6, SEQ ID 5 NO:8 and/or SEQ ID NO:10 or mutants or derivatives or further splice variants thereof.

Another embodiment provides a recombinant molecule having the following characteristics:

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

15 Another embodiment provides a recombinant molecule having the following characteristics:

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

- 25 (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

30

Another embodiment provides a recombinant molecule having the following characteristics:

- 6 -

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- 5 (ii) exhibits at least one biological property in common with VEGF.

Such properties of VEGF include at least one of:

- (a) ability to induce proliferation of vascular endothelial cells;
- (b) an ability to interact with *flt-1/flk-1* family of receptors;
- 10 (c) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

In accordance with the present invention, a preferred similarity is at least about 40%, more preferably at least about 50% and even more preferably at least about 65%
15 similarity.

Still a further aspect of the present invention contemplates a peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a splice variant thereof such as set forth in SEQ ID NO:6, SEQ ID NO:8 or SEQ ID
20 NO:10 or a chemical equivalent thereof. The biologically isolated or recombinant molecule of the present invention may be naturally glycosylated or may comprise an altered glycosylation pattern depending on the cells from which it is isolated or synthesised. For example, if produced by recombinant means in prokaryotic organisms, the molecule would be non-glycosylated. The molecule may be a full length, naturally
25 occurring form or may be a truncated or otherwise derivatised form.

Yet another aspect of the present invention is directed to a nucleic acid molecule encoding the VEGF-like molecule herein described. More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides
30 substantially as set forth in SEQ ID NO:3 or having at least 15% similarity to all or part thereof or being capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the

- 7 -

nucleic acid sequence having at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence as set forth in SEQ ID NO:3. The nucleotide sequence set forth in SEQ ID NO:3 is also referred to herein as "SOM175". Preferably, the percentage dissimilarity is about 35%, more preferably about 39% and even more preferably about 5 40-50% or greater.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (1989) at pages 9.47-9.51 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is 10 defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at ≥ 45°C for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v 15 SDS at 60°C for 1-3 hours.

The present invention further contemplates a nucleic acid molecule which encodes a VEGF-like molecule as hereinbefore described having at least 15% nucleotide sequence homology to SEQ ID NO:3. Preferred levels of homology include at least about 40%. 20 more preferably around 60-70%.

The present invention is further directed to the murine homologue of human VEGF (referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human 25 VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in Figure 9.

The VEGF-like molecule of the present invention will be useful in the development of a range of therapeutic and/or diagnostic applications alone or in combination with other 30 molecules such as VEGF. The present invention extends, therefore, to pharmaceutical compositions comprising the VEGF-like molecule or parts, fragments, derivatives, homologues or analogues thereof together with one or more pharmaceutically acceptable

- 8 -

carriers and/or diluents. Furthermore, the present invention extends to vectors comprising the nucleic acid sequence set forth in SEQ ID NO:3 or having at least about 15%, more preferably about 40% and even more preferably around 60-70% similarity thereto but at least 30% and more preferably around 39% dissimilarity thereto and host 5 cells comprising same. In addition, the present invention extends to ribozymes and antisense molecules based on SEQ ID NO:3 as well as neutralizing antibodies to the VEGF-like molecule. Such molecules may be useful in ameliorating the effects of, for example, over expression of VEGF-like genes leading to angiogenesis or vascularization of tumours.

10

Another aspect of the present invention contemplates a method of inducing astroglial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 15 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial 20 proliferation.

Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:6.

25 A further aspect of the present invention provides a method of promoting neural survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 30 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

- 9 -

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence

5 set forth in SEQ ID NO:3 or SEQ ID NO:6.

The present invention also contemplates antibodies to the VEGF-like molecule or nucleic acid probes to a gene encoding the VEGF-like molecule which are useful as diagnostic agents.

10

The present invention is further described by reference to the following non-limiting Figures and/or Examples.

In the Figures:

15

Figure 1 Nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence [SEQ ID NO:2] of VEGF₁₆₅.

20

Figure 2 Nucleotide sequence [SEQ ID NO:3] and corresponding amino acid sequence [SEQ ID NO:4] of SOM175.

Figure 3 Results of BLAST search with SOM175 protein sequence.

25

Figure 4 BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

Figure 5 Multiple alignment of VEGF₁₆₅ with SOM175 and its splice variants at the nucleotide level.

30

Figure 6 Multiple alignment of VEGF₁₆₅ with SOM175 and its splice variants at the amino acid level.

Figure 7 Diagrammatic representation of SOM175 and its splice variants.

- 10 -

Figure 8(a) Diagrammatic representation of genomic structure of human SOM175 genomic showing exon/intron map.

Figure 8(b) Diagrammatic representation of genomic structure of human SOM175

5 showing exon/intron boundaries.

Figure 9 Nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting from the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue 10 of the predicted mature protein after the putative signal peptide has been removed. The alternately spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of mVRF₁₆₇ and mVRF₁₈₆ are underlined and a polymorphic AC repeat in the 3' UTR is indicated by a stippled box. The positions of intron/exons 15 boundaries are indicated by arrowheads.

Figure 10 BESTFIT alignments of human and murine VRF protein isoforms. A: mVRF₁₆₇ and hVRF₁₆₇. B: mVRF₁₈₆ and hVRF₁₈₆ from the point where the sequences diverge from the respective 167 amino acid isoforms. Amino acid identities are marked 20 with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

Figure 11 BESTFIT alignment of mVRF₁₆₇ and mVEGF₁₈₈ (Breier *et al*, 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. 25 Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

Figure 12 Comparison of gene structure between VRF (a generic VRF gene is shown since the intron/exon organisation of the mouse and human homologues is almost 30 identical) and other members of the human VEGF/PIGF/PDGF gene family. Exons are represented by boxes. Protein coding regions and untranslated regions are shown by filled and open sections respectively. The hatched region in VRF indicates the

- 11 -

additional 3' UTR sequence formed by alternate splicing of the VRF₁₈₆ isoform. Potential alternate splice products of each gene are shown.

5 **Figure 13** Autoradiogram of a Northern blot of total RNA from various adult mouse tissues (as indicated) hybridised with an mVRF cDNA clone. A major transcript of 1.3 kb was detected in all samples.

10 **Figure 14** Film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) 15 and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled. The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an 20 excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

25 **Figure 15** Dark - (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle (M in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toluidine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not 30 shown) where largely positive for mVRF mRNA. Scale bars = 0.1 mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

- 12 -

Figure 16 Effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival, % neurite outgrowth and average neurite length (μm).

Figure 17 Effects of VEGF and SOM175 on chick glia. Tested were CNS glial,
5 peripheral glia and CNS oligodendrocytes.

Figure 18 Effect of various SOM175 proteins on mouse astroglial cells. ■ ^3H (cpm)

1. FGF-2 (10 ng/ml) positive control
2. SOM Δ X6* 1 ng/ml
- 10 3. SOM Δ X6 10 ng/ml
4. SOM Δ X6 100 ng/ml
5. SOM Δ X6 1000 ng/ml
6. SOM Δ X6 1000 ng/ml, no heparin
7. SOMX6** 1 ng/ml
- 15 8. SOMX6 10 ng/ml
9. SOMX6 100 ng/ml
10. SOMX6 1000 ng/ml
11. SOMX6 1000 ng/ml, no heparin

* This refers to SOM175 absent exon 6;

20 ** This refers to SOM175.

Figure 19 Effect of various SOM175 proteins on mouse oligodenroglial cells. ■ ^3H (cpm)

1. FGF-2 (10 ng/ml) positive control
- 25 2. SOM Δ X6* 1 ng/ml
3. SOM Δ X6 10 ng/ml
4. SOM Δ X6 100 ng/ml
5. SOM Δ X6 1000 ng/ml
6. SOM Δ X6 1000 ng/ml, no heparin
- 30 7. SOMX6** 1 ng/ml
8. SOMX6 10 ng/ml
9. SOMX6 100 ng/ml

- 13 -

10. SOMX6 1000 ng/ml
11. SOMX6 1000 ng/ml, no heparin

* This refers to SOM175 absent exon 6;

** This refers to SOM175.

5

Figure 20 Effect of various SOM175 proteins on mouse forebrain neurons. ■ % survival

1. FGF-2 (10 ng/ml) positive control
2. SOM Δ X6* 1 ng/ml
3. SOM Δ X6 10 ng/ml
4. SOM Δ X6 100 ng/ml
5. SOM Δ X6 1000 ng/ml
6. SOM Δ X6 1000 ng/ml, no heparin
7. SOMX6** 1 ng/ml
8. SOMX6 10 ng/ml
9. SOMX6 100 ng/ml
10. SOMX6 1000 ng/ml
11. SOMX6 1000 ng/ml, no heparin

* This refers to SOM175 absent exon 6;

20 ** This refers to SOM175.

TABLE 1
SUMMARY OF SEQUENCE IDENTITY NUMBERS

| | | |
|----|--------------|--|
| 5 | SEQ ID NO:1 | Nucleotide sequence of VEGF ₁₆₅ |
| | SEQ ID NO:2 | Amino acid sequence of VEGF ₁₆₅ |
| | SEQ ID NO:3 | Nucleotide sequence of SOM175 (VEGF-like molecules) |
| | SEQ ID NO:4 | Amino acid sequence of SOM175 |
| 10 | SEQ ID NO:5 | Nucleotide sequence of SOM175 absent exon 6 |
| | SEQ ID NO:6 | Amino acid sequence of SOM175 absent exon 6 |
| | SEQ ID NO:7 | Nucleotide sequence of SOM175 absent exon 6 and exon 7 |
| | SEQ ID NO:8 | Amino acid sequence of SOM175 absent exon 6 and exon 7 |
| | SEQ ID NO:9 | Nucleotide sequence of SOM175 absent exon 4 |
| 15 | SEQ ID NO:10 | Amino acid sequence of SOM175 absent exon 4 |
| | SEQ ID NO:11 | Oligonucleotide |
| | SEQ ID NO:12 | Oligonucleotide |
| | SEQ ID NO:13 | Oligonucleotide |
| | SEQ ID NO:14 | Oligonucleotide |
| 20 | | |

EXAMPLE 1

Human cDNA clones

The original SOM175 cDNA was isolated by screening a human foetal brain library 25 (λ zapII, Stratagene) with the cosmid D11S750 (Larsson *et al.*, 1992). The plasmid was excised "in vivo" and a single 1.1kb cDNA was obtained. Three independent SOM175 cDNAs clones were also isolated from a human foetal spleen library (Stratagene, Uni-zap) using the above-mentioned SOM175 insert as a probe. Three clones were obtained: SOM175-4A, -5A and -6A. SOM175-5A is an alternately spliced clone with exon 4 30 being absent (SOM175-e4). These library screens were performed using hybridisation conditions recommended by the manufacturer of the library (Stratagene) and random primed insert of SOM175.

- 15 -

Two partial human SOM175 cDNAs have also isolated from a λGT11 human melanoma cell line A2058 library (Clontech) cDNA library screens were performed using hybridisation conditions described by Church and Gilbert, 1984). In each case, the probe was generated by random priming of a PCR product derived from SOM175 (18f-
5 700r).

Mouse cDNA Clones

Human SOM175 was also used to screen a mouse neonatal whole brain cDNA library (Unizap, Stratagene). Four non-chimeric clones were isolated: M175-A, B, C, D. All
10 clones were partial cDNAs and M175-C contained several introns. Three of these cDNAs lacked the exon 6.

Another clone referred to as M1 was completely sequenced and was found to contain the full open reading frame plus part of the 5'utr and total 3'utr.
15

EXAMPLE 2

DNA SEQUENCE ANALYSIS

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in Figure 2 with its corresponding amino acid sequence. This sequence was screened for
20 open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see Figure 2). There appears to be little 5' untranslated sequences (2bp). The 3' untranslated region appears to be complete as it includes a poly-adenylation signal and poly-A tail.

25 Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see Figure 3). The amount of homology between SOM175 and human VEGF₁₆₅ was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures 4 and 5). Nucleotide homology was estimated at 69.7% and protein homology was
30 estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams *et al.*, 1993).

- 16 -

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47,
5 Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in Figure 6. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that
10 SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of VEGF₁₆₅ is shown in Figure 1.

EXAMPLE 3

The percentage similarity and divergence between VEGF₁₆₅ family and SOM175 family
15 (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abbreviated to SOM715-e6 where all of exon 6 is deleted; SOM715-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic
20 maps of SOM175 showing intron/exon boundaries are shown in Figure 8a and 8b.

Table 2.1

**A Percent nucleotide similarity between splice variants of SOM175 and
5 human VEGF₁₆₅**

| | VEGF ₁₆₅ | SOM175 | SOM175-e6 | SOM175-e6&7 | SOM175-e4 |
|---------------------|---------------------|--------|-----------|-------------|-----------|
| VEGF ₁₆₅ | *** | 34.9 | 39.7 | 41.4 | 37.0 |
| 10 SOM175 | | *** | 98.9 | 95.1 | 99.2 |
| SOM175-e6 | | | *** | 98.8 | 84.0 |
| SOM175-e6&7 | | | | *** | 80.3 |
| SOM175-e4 | | | | | *** |

15

- 18 -

B Percent nucleotide divergence between splice variants of SOM175 and human VEGF₁₆₅

| | | VEGF ₁₆₅ | SOM175 | SOM175-e6 | SOM175-e6&7 | SOM175-e4 |
|----|---------------------------|---------------------|--------|-----------|-------------|-----------|
| | VEGF₁₆₅ | *** | 41.7 | 41.6 | 41.7 | 41.8 |
| | SOM175 | | *** | 0.2 | 0.2 | 0.0 |
| | SOM175-e6 | | | *** | 0.0 | 0.2 |
| 10 | SOM175-e6&7 | | | | *** | 0.3 |
| | SOM175-e4 | | | | | *** |

Table 2.2

A Percent amino acid identity between splice variants of SOM175 and human VEGF₁₆₅

| | | VEGF ₁₆₅ | SOM175 | SOM175-e6 | SOM175-e6&7 | SOM175-e4 |
|----|---------------------------|---------------------|--------|-----------|-------------|-----------|
| 20 | VEGF₁₆₅ | *** | 31.4 | 42.3 | 33.5 | 40.6 |
| | SOM175 | | *** | 74.7 | 73.7 | 99.1 |
| | SOM175-e6 | | | *** | 76.8 | 99.1 |
| | SOM175-e6&7 | | | | *** | 99.1 |
| | SOM175-e4 | | | | | *** |

25

- 19 -

B Percent amino acid divergence between splice variants of SOM175 and human VEGF₁₆₅

| | VEGF ₁₆₅ | SOM175 | SOM175-e6 | SOM175-e6&7 | SOM175-e4 |
|---------------------|---------------------|--------|-----------|-------------|-----------|
| 5 | | | | | |
| VEGF ₁₆₅ | *** | 65.7 | 55.4 | 54.6 | 57.4 |
| SOM175 | | *** | 19.9 | 4.2 | 0.0 |
| SOM175-e6 | | | *** | 0.0 | 0.0 |
| 10 SOM175-e6&7 | | | | *** | 0.0 |
| SOM175-e4 | | | | | *** |

15

EXAMPLE 4

BIOASSAYS TO DETERMINE THE FUNCTION OF SOM175

Assays are conducted to evaluate whether SOM175 has similar activities to VEGF on endothelial cell function, angiogenesis and wound healing. Other assays are performed based on the results of receptor binding distribution studies.

20

Assays of endothelial cell function

Endothelial cell proliferation. Endothelial cell growth assays as described in Ferrara & Henzel (1989) and in Gospodarowicz *et al* (1989).

25 *Vascular permeability assay.* This assay, which utilises the Miles test in guinea pigs, will be performed as described in Miles & Miles (1952).

Cell adhesion assay. The influence of SOM175 on adhesion of polymorphs to endothelial cells is analysed.

30

Chemotaxis. This is performed using the standard Boyden chamber chemotaxis assay.

- 20 -

Plasminogen activator assay. Endothelial cells are tested for plasminogen activator and plasminogen activator inhibitor production upon addition of SOM175 (Pepper *et al* (1991)).

- 5 *Endothelial cell migration assay.* The ability of SOM175 to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al* (1986).

Angiogenesis Assay

- SOM175 induction of an angiogenic response in chick chorioallantoic membrane is
10 evaluated as described in Leung *et al* (1989).

Possible neurotrophic actions of SOM175 are assessed using the following assays:

Neurite outgrowth assay and gene induction (PC12 cells)

- 15 PC12 cells (a phaeochromocytoma cell line) respond to NGF and other neurotrophic factors by developing the characteristics of sympathetic neurons, including the induction of early and late genes and the extension of neurites. These cells are exposed to SOM175 and their response monitored (Drinkwater *et al* (1991); and Drinkwater *et al* (1993)).

20

Cultured neurons from the Peripheral Nervous System (PNS)

Primary cultures of the following PNS neurons are exposed to SOM175 and monitored for any response:

- sensory neurons from neural crest and dorsal root ganglia
- sympathetic neurons from sympathetic chain ganglia
- placode derived sensory neurons from nodose ganglia
- motoneurons from spinal cord

The assays are described in Suter *et al* (1992) and in Marinou *et al* (1992).

- 30 Where an *in vitro* response is observed, *in vivo* assays for properties such as uptake and retrograde transport are performed as described in Hendry *et al* (1992).

- 21 -

Nerve regeneration (PNS)

Where neurotrophic effects of SOM175 are observed, its possible role in the regeneration of axotomised sensory neurons, sympathetic neurons and motoneurons is analysed by the methods of Otto *et al* (1989); Yip *et al* (1984) and Hendry *et al*

5 (1976).

Actions of SOM175 on CNS neurons

The ability of SOM175 to promote survival of central nervous system neurons is

analysed as described in Hagg *et al* (1992); Williams *et al* (1986); Hefti (1986) and

10 Kromer (1987).

Wound Healing

The ability of SOM175 to support wound healing are tested in the most clinically

relevant model available, as described in Schilling *et al* (1959) and utilised by Hunt

15 *et al* (1967).

The Haemopoietic System

A variety of *in vitro* and *in vivo* assays on specific cell populations of the haemopoietic system are available and are outlined below:

20 *Stem Cells*

Murine

A variety of novel *in vitro* murine stem cell assays have been developed using FACS-purified cells:

25 (a) **Repopulating Stem Cells**

These are cells capable of repopulating the bone marrow of lethally irradiated mice, and have the Lin⁻, Rh^{hi}, Ly-6A/E⁺, c-kit⁺ phenotype. The test substance is tested on these cells either alone, or by co-incubation with multiple factors, followed by measurement of cellular proliferation by ³H thymidine incorporation.

30

(b) Late Stage Stem Cells

- These are cells that have comparatively little bone marrow repopulating ability but can generate D13 CFU-S. These cells have the Lin⁻, Rh^{hi}, Ly-6A/E⁺, c-kit⁺ phenotype. The test substance is incubated with these cells for a period of time, injected into lethally irradiated recipients, and the number of D13 spleen colonies enumerated.
- 5

(c) Progenitor-Enriched Cells

- 10 These are cells that respond *in vitro* to single growth factors, and have the Lin⁻, Rh^{hi}, Ly-6A/E⁺, c-kit⁻ phenotype. This assay will show if SOM175 can act directly on haemopoietic progenitor cells. The test substance is incubated with these cells in agar cultures, and the number of colonies enumerated after 7-14 days.

15 **Atherosclerosis**

- Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change in their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell. An *in vitro* assay that measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment is used to assess the effect of SOM175 on smooth muscle cells. The system uses a modified Rose chamber in which different cell types are seeded onto opposite coverslips.
- 20

25

Effects of SOM175 on bone

- The ability of SOM175 to regulate proliferation of osteoblasts is assayed as described in Lowe *et al* (1991). Any effects on bone resorption are assayed as described in Lowe *et al* (1991). Effects on osteoblast migration and changes in intracellular molecules (e.g. cAMP accumulation, alkaline phosphatase levels) are analysed as described in Midy *et al* (1994).
- 30

- 23 -

Effects on skeletal muscle cells

Effects of SOM175 on proliferation of myoblasts and development of myotubes can be determined as described by Ewton *et al* (1980) and by Gospodarowicz *et al* (1976).

5

EXAMPLE 5

CLONING MURINE VEGF DNA

Isolation of cDNAs

Murine VRF (mVRF) clones were selected from a lambda Zap new born whole brain
10 cDNA library (Stratagene). Primary phage from high density filters (5×10^4 pfu/plate) were identified by hybridisation with a 682bp ^{32}P -labelled probe generated by PCR from an hVRF cDNA (pSOM175) as described above. Hybridisation and stringent washes of nylon membranes (Hybond-N) were carried out at 65°C under conditions described by Church and Gilbert (1984). Positive plaques were picked,
15 purified and excised *in vivo* to produce bacterial colonies containing cDNA clones in pBluescript SK-.

Isolation of genomic clones

Genomic clones were isolated from a mouse strain SV/129 library cloned in the
20 lambda Fix II vector (Stratagene). High density filters (5×10^4 pfu/filter) were screened with a 563 bp ^{32}P -labelled probe generated by PCR amplification of the nucleotide 233-798 region of the mVRF cDNA (see Figure 9). Positive clones were plugged and re-screened with filters containing 400-800 pfu. Large scale phage preparations were prepared using the QIAGEN lambda kit or by ZnCl₂ purification
25 (Santos, 1991).

Nucleotide sequencing and analysis

cDNAs were sequenced on both strands using a variety of vector-based and internal primers with Applied Biosystems Incorporated (ABI) dye terminator sequencing kits
30 according to the manufacturer's specifications. Sequences were analysed on an ABI Model 373A automated DNA sequencer. Peptide homology alignments were performed using the program BESTFIT (GCG, Wisconsin).

- 24 -

Identification of intron/exon boundaries

- Identification of exon boundaries and flanking regions was carried out using PCR with mouse genomic DNA or mVRF genomic lambda clones as templates. The primers used in PCR to identify introns were derived from the hVRF sequence and to
- 5 allow for potential human-mouse sequence mismatches annealing temperatures 5-10°C below the estimated T_m were used. All PCR products were sized by agarose gel electrophoresis and gel purified using QIAquick spin columns (Qiagen) and the intron/exon boundaries were sequenced directly from these products. In addition, some splice junctions were sequenced from subcloned genomic fragments of MVRF.
- 10 Intron/exon boundaries were identified by comparing cDNA and genomic DNA sequences.

Northern analysis

- Total cellular RNA was prepared from a panel of fresh normal adult mouse tissues
- 15 (brain, kidney, liver, muscle) using the method of Chomczynski and Sacchi (1987). 20 μ g of total RNA were electrophoresed, transferred to a nylon membrane (Hybond N, Amersham) and hybridised under standard conditions (Church & Gilbert, 1984). Filters were washed at 65°C in 0.1xSSC (20xSSC is 3M NaCl/0.3M trisodium citrate), 0.1% SDS and exposed to X-ray film with intensifying screens at -70°C for
- 20 1-3 days.

Characterisation of mVRF cDNAs

- Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered
- 25 and sequenced. The cDNA sequences were complied to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR (Figure 9).

- 30 The predicted initiation codon matched the position of the start codon in hVRF. One other out of frame ATG was located at position -47 and two termination codons were observed upstream (positions -9 and -33, respectively) and in-frame with the putative

- 25 -

initiation codon.

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage within mVRF is expected
5 to occur after residue 21 (Figure 10). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and
10 lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms (Figure 10).

The message encoding mVRF₁₈₆ contains a 621 bp ORF with coding sequences
15 terminating at position +622, towards the end of exon 7 (Figure 9). The smaller message encoding mVRF₁₆₇ actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a stop codon (TGA) at position +666, near the beginning of exon 8 (Figure 9).

20

The mVRF₁₈₆ protein has strong homology to the amino and central portions of VEGF while the carboxyl end is completely divergent and is alanine rich. mVRF₁₆₇ possesses these similarities and also maintains homology to mVEGF right through to the C-terminus (Figure 11). The overall homology of mVRF₁₆₇ to hVRF₁₆₇ was
25 85% identity and 92% similarity, respectively (Figure 10). Likewise, homology between mVRF₁₆₇ and mVEGF (Breier *et al*, 1992) was 49% identity and 71% conservative amino acid substitution, respectively (Figure 11).

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel *et al*, 1986) was
30 not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs (Figure 9). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the

- 26 -

extreme 3' end of the 3' UTR (nucleotide positions 998 to 1011, Figure 9).

Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

5 Genomic characterisation of mVRF

Intron/exon boundaries (Table 3) were mapped using primers which flanked sequences homologous to the corresponding hVRF boundaries. Introns I, III, IV and VI of mVRF (Table 3, Figure 12) were smaller than the hVRF intervening sequences. The complete genomic sequence was compiled from the 5' UTR of 10 mVRF through to intron VI, the largest intervening region (2.2 kb), by sequencing amplified introns and cloned genomic portions of mVRF. There was only one major difference in genomic structure between mVRF and hVRF and that was the exon 7/intron VI boundary of mVRF was located 10bp further downstream in relation to the cDNA sequence, hence exon 7 in mVRF is 10bp longer than the corresponding 15 exon in hVRF.

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF (Figure 10) suggests that this sequence is not a retained intronic sequence but rather 20 encodes a functional part of the VRF₁₈₆ isoform.

General intron/exon structure is conserved between the various members of the VEGF gene family (VEGF, PIGF, hVRF) and therefore it is not surprising that the overall genomic organisation of the mVRF gene is very similar to these genes 25 (Figure 12).

Previous comparative mapping studies have shown that the region surrounding the human multiple endocrine neoplasia type 1 disease locus on chromosome 11q13 is syntenic with the proximal segment of mouse chromosome 19 (Rochelle *et al*, 1992). 30 Since the inventors have mapped the hVRF gene to within 1kb of the human *MEN1* locus (see above) it is most likely that the murine VRF gene maps near the centromere of chromosome 19.

- 27 -

Expression studies of mVRF

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiquitous and occurs primarily as a major band of approximately 1.3kb in size (Figure 14). This is somewhat different to the 5 pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a difference in the length of the respective 5' UTRs.

10

EXAMPLE 6

EXPRESSION OF MURINE VEGF IN PRE- AND POST-NATAL MOUSE

Animals

Timed pregnant (n=4) and young adult (n=2) mice (C57 inbred strain, ALAB, Sweden) were sacrificed with carbon dioxide, and the relevant tissues were taken out 15 and frozen on a chuck. Tissues were kept at -70°C until further use. Two gestational ages was used in this study; embryonic day 8 (E8), 14 and E17.

In situ hybridisation histochemistry

In situ hybridisation was performed as previously described (Dagerlind *et al*, 1992).

20 Briefly, transverse sections (14µm) were cut in a cryostat (Microm, Germany), thawed onto Probe-On slides (Fisher Scientific, USA) and stored in black sealed boxes at -70°C until used. The sequences of the synthetic 42-mer oligonucleotides complementary to mRNA encoding mVRF were
ACCACCACCTCCCTGGGCTGGCATGTGGCACGTGCATAAACG [SEQ ID
25 NO:11] (complementary to nt 120-161) and
AGTTTTGACCACATTGCCATGAGTTCCATGCTCAGAGGC [SEQ ID
NO:12] (complementary to nt 162-203). To detect the two alternative splice forms oligonucleotide GATCCTGGGCTGGAGTGGATGGATGATGTCAGCTGG [SEQ ID NO:13] (complementary to nt xxx-xxx) and
30 GCGGGCAGAGGATCCTGGGCTGTCTGGCCTCACAGCACT [SEQ ID NO:14] were used. The probes were labeled at the 3'-end with deoxyadenosine-alpha[thio]triphosphate [35 S] (NEN, USA) using terminal deoxynucleotidyl

- 28 -

transferase (IBI, USA) to a specific activity of $7\text{-}10 \times 10^8$ cpm/ μg and hybridised to the sections without pretreatment for 16-18 h at 42°C. The hybridisation mixture contained: 50% v/v formamide, 4 x SSC (1 x SSC = 0.15M NaCl and 0.015M sodium-citrate), 1 x Denhardt's solution (0.02% each of polyvinyl-pyrrolidone, BSA 5 and Ficoll), 1% v/v sarcosyl (N-lauroylsarcosine; Sigma), 0.02M phosphate buffer (pH 7.0), 10% w/v dextran sulfate (Pharmacia, Sweden), 250 $\mu\text{g}/\text{ml}$ yeast tRNA (Sigma), 500 $\mu\text{g}/\text{ml}$ sheared and heat denatured salmon sperm DNA (Sigma) and 200mM dithiothreitol (DTT; LKB, Sweden). In control sections, the specificity of both probes was checked by adding a 20-fold excess of unlabeled probe to the 10 hybridisation mixture. In addition, adjacent sections were hybridised with a probe unrelated to this study which gave a different expression pattern. Following hybridisation the sections were washed several times in 1 x SSC at 55°C, dehydrated in ethanol and dipped in NTB2 nuclear track emulsion (Kodak, USA). After 3-5 weeks the sections were development in D-19 developer (Kodak, USA) and cover-slipped. In some cases, sections were opposed to an autoradiographic film (Beta-max 15 autoradiography film Amersham Ltd, UK) prior to emulsion-dipping.

The four different probes gave identical hybridisation patterns in all tissues examined. Mouse VRF expression was detecting already in the E8 embryo, in which positive 20 signal was recorded over structures most likely corresponding to the neuronal tube. In sagittal sections of E14 mouse embryo the strongest hybridisation signal was present over heart and in the nervous system, especially cerebral cortex (Figure 14A). A low level of expression was present in all other tissues. At a later gestational age, E17, a high mVRF mRNA signal was confined to the heart and brown fat tissue in 25 the back and around the neck (Figure 14B). Clearly positive hybridisation signals were present in the gray of the spinal cord and in the tongue (Figure 14B). Expression in the cerebral cortex was clearly reduced compared to day 14. The weak background expression seen in the E14 embryo in for example muscle, had decreased at this gestational age. A strong mVRF mRNA hybridisation signal was present 30 solely over the heart and in the brown fat in the young adult mice (Figure 14C). The signal over the heart was evenly distributed over the entire ventricular wall, including the papillary muscles (Figure 14D). In sections of heart tissue hybridised with an

- 29 -

excess of cold probe, no specific labeling over background signal was recorded (Figure 14E).

Apart from the heart, mVRF mRNA signal was present over certain tissues on the
5 outside of the thoracic cage that morphologically resembled brown fat. This was verified with sudan black counterstaining, which showed a strong staining in the same areas (Figure 15A and 15B). In transverse sections of adult mouse spinal cord, the mVRF probes gave a neuronal staining pattern over the gray matter (Figure 15C). Counterstaining with toluidine (Figure 15D) showed that motoneurons in the ventral
10 horn (Figure 15C and 15D), interneurons (Figure 15C) in the deep part of the dorsal horn and around the central canal were to a large extent positive for mVRF mRNA.

EXAMPLE 7

EFFECTS OF VEGF AND SOM175 PROTEINS ON CHICK 15 SENSORY NEURONS

The effects of VEGF and SOM175 proteins on embryonic day 8 chick sensory neurons were determined using the method of Nurcombe *et al* (1992). The neuronal assay was read at 48 hours using 2000 cells per assay well. The results were obtained using ^3H -thymidine counts. The percentage survival of neurons, neurite
20 outgrowth and average neurite length in μm were determined using NGF as positive control and various concentrations of VEGF, VEGF in the presence of heparin and VEGF in the presence of heparin and 5 μM , 5'-flurouracil (5FU). 5FU kills glial cells.
25 The results are shown in Figure 16. The results show that VEGF is effective in promoting neuronal survival but that this requires the presence of glial cells. Figure 17 shows the results of the effect of VEGF and SOM175 on three types of chick glia. The glia tested were CNS glia, peripheral glia and CNS oligodendrocytes. Heparin was used as 10 $\mu\text{g}/\text{ml}$ in all cultures and the assay was read at 24 hours.
30 Results were measured in ^3H -thymidine counts using 2000 cells per well.

The results show that for chick central and peripheral neurons, astroglia were markedly stimulated to proliferate by SOM175 in the presence of heparin but that chick oligodendrocytes showed negligible increase in the rate of division.

5

EXAMPLE 8

EFFECTS OF SOM175 PROTEINS ON MOUSE PRIMARY AND CENTRAL NEURONS

The results in Example 7 show that VEGF isoform had an effect on chick primary 10 and central neurons through the agency of the astroglial cells. Similar experiments were repeated in mouse cells.

Culture conditions

Neuronal and glial cells for all *in vitro* experiments were prepared and cultured 15 according to the techniques described in "Methods in Neurosciences (Vol. 2): Cell Culture" Ed. P.M. Conn, Academic Press, San Diego, 1990, pp33-46 for astroglial cells, pp56-74 for oligodendroglial cells, and pp87-102 for central neurons.

Cells were plated onto 24-well culture clusters (Nunc) coated with poly-L-ornithine 20 (0.1 mg/ml, 1h) at a density of 2,000 cells/well. After 48 hours in culture, neurons were counted in the wells under inverted phase light using well established techniques (Maruta *et al.* 1993) and glial cells assessed with [³H]thymidine uptake to monitor cell division rates as below. Heparin (10 μ g/ml, low molecular weight fraction, Sigma Chemical Corp.) was present at all times in the culture media except 25 where noted. The neuronal cultures were supplemented with 5mM 5-fluoro-2-deoxyuridine (Sigma) to suppress background glial growth.

³H-Thymidine incorporation assay for glial cell proliferation

The cells were pulsed for 14h with ³H-thymidine (specific activity 103 μ Ci/ug) from 30 a stock concentration of 0.1 mCi/ml in standard medium, giving a final incubating volume of 20 μ l/well. The contents of the wells were harvested and absorbed onto nitrocellulose paper (Titertek, Flow). Remaining adherent cells were removed by

- 31 -

incubation with trypsin/versene (CSL Limited, Victoria, Australia) for 5 min. This procedure was carried out twice. The nitrocellulose discs were washed in a standard Titertek harvester (Flow) using first distilled water, and then methanol. The nitrocellulose discs were dried, scintillation fluid (containing 5% v/v Triton-X) added
5 and the discs counted on a scintillation counter.

Greatest activity was seen with preparations of SOM175 absent exon 6 (SOM Δ X6) on mouse astroglial cell cultures, where there was a significant stimulus to their proliferation when delivered in conjunction with heparin (Figure 16). Little stimulus
10 was given to the proliferation of oligodendroglial cells (Figure 17), and very little discernable potentiation of the survival response of isolated forebrain neurons (Figure 18). The standard deviation on all three graphs for each point was less than 8%.

The viability of neurons can be maintained by promoting glial cell proliferation.
15 Furthermore, SOM Δ X6 is a good inducer of astroglial proliferation and may be expressed in conjunction with the formation of astroglial endfeet on central nervous system endothelial cells.

Those skilled in the art will appreciate that the invention described herein is
20 susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

- 32 -

TABLE 3
Splice junctions of the murine VRF gene

| | | | | | |
|----|-----------------|---------------|------------------|-------------|---------|
| 5 | 5' UTR* | Exon 1 >223bp | CCCAAGgtacgtgcgt | Intron I | 495bp |
| | ttccccacagGCC | Exon 2 43bp | GAAAGgtataatag | Intron II | 288bp |
| | ctgcccacagTGG | Exon 3 197bp | TGCAGgtaccagggc | Intron III | 196bp |
| | ctgagcacagATC | Exon 4 74bp | TGCAGgtgccagccc | Intron IV | 182bp |
| | ctctttcagACCTA | Exon 5 36bp | GACAGAttcttggtg | Intron V | 191bp |
| 10 | ctcctcttagGGTTG | Exon 6 101bp | | (no intron) | |
| | CCCACTCCAGCCCCA | Exon 7 135bp | TGTAGgttaaggagtc | Intron VI | ~2200bp |
| | cactccccagGTGCC | Exon 8 394bp | AGAGATGGAGACACT | | |

Uppercase and lowercase letters denote exonic and intronic sequences respectively.

15 * Indicates that the 5' end of exon 1 has not yet been determined.

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- 36 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(countries other than US) AMRAD OPERATIONS PTY. LTD.
(us only) Hayward, N and Weber, G

(ii) TITLE OF INVENTION: A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

(iii) NUMBER OF SEQUENCES: 14

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 22-FEB-1996

(vii) PRIOR APPLICATION DATA:

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(B) FILING DATE: 20-NOV-1995
(A) APPLICATION NUMBER: AU PN7274
(B) FILING DATE: 22-DEC-1995

- 37 -

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 649 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 17...589

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCGGGCCTCC GAAACC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC 49
 Met Asn Phe Leu Leu Ser Trp Val His Trp Ser
 1 5 10

CTT GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA 97
 Leu Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala
 15 20 25

CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC 145
Pro Met Ala Glu Gly Gly Gln Asn His His Glu Val Val Lys Phe
30 35 40

ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG 193
 Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val
 45 50 55

GAC ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA 241
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
60 65 70 75

TCC TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC 289
 Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly
 80 85 90

- 38 -

| | | | |
|---|------------|-----|-----|
| CTG GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG | 337 | | |
| Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met | | | |
| 95 | 100 | 105 | |
| CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA | 385 | | |
| Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu | | | |
| 110 | 115 | 120 | |
| CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA | 433 | | |
| Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln | | | |
| 125 | 130 | 135 | |
| GAA AAT CCC TGT GGG CCT TGC TCA GAG CGG AGA AAG CAT TTG TTT GTA | 481 | | |
| Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val | | | |
| 140 | 145 | 150 | 155 |
| CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT | 529 | | |
| Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg | | | |
| 160 | 165 | 170 | |
| TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC | 577 | | |
| Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp | | | |
| 175 | 180 | 185 | |
| AAG CCG AGG CGG TGAGCCGGGC AGGAGGAAGG AGCCTCCCTC AGCGTTTCGG | 629 | | |
| Lys Pro Arg Arg | | | |
| 190 | | | |
| GAACCAGATC TCTCACCAAGG | 649 | | |

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 191 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | | | |
|---|----|----|----|
| Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu | | | |
| 1 | 5 | 10 | 15 |
| Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly | | | |
| 20 | 25 | 30 | |
| Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln | | | |
| 35 | 40 | 45 | |
| Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu | | | |
| 50 | 55 | 60 | |

- 39 -

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Pro | Asp | Glu | Ile | Glu | Tyr | Ile | Phe | Lys | Pro | Ser | Cys | Val | Pro | Leu |
| 65 | | | | 70 | | | | | 75 | | | | | 80 | |
| Met | Arg | Cys | Gly | Gly | Cys | Cys | Asn | Asp | Glu | Gly | Leu | Glu | Cys | Val | Pro |
| | | | 85 | | | | | | 90 | | | | | 95 | |
| Thr | Glu | Glu | Ser | Asn | Ile | Thr | Met | Gln | Ile | Met | Arg | Ile | Lys | Pro | His |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Gln | Gly | Gln | His | Ile | Gly | Glu | Met | Ser | Phe | Leu | Gln | His | Asn | Lys | Cys |
| | | | 115 | | | | 120 | | | | | 125 | | | |
| Glu | Cys | Arg | Pro | Lys | Lys | Asp | Arg | Ala | Arg | Gln | Glu | Asn | Pro | Cys | Gly |
| | | | 130 | | | 135 | | | | 140 | | | | | |
| Pro | Cys | Ser | Glu | Arg | Arg | Lys | His | Leu | Phe | Val | Gln | Asp | Pro | Gln | Thr |
| | | | 145 | | | 150 | | | | 155 | | | | 160 | |
| Cys | Lys | Cys | Ser | Cys | Lys | Asn | Tnr | Asp | Ser | Arg | Cys | Lys | Ala | Arg | Gln |
| | | | | 165 | | | | | 170 | | | | 175 | | |
| Leu | Glu | Leu | Asn | Glu | Arg | Thr | Cys | Arg | Cys | Asp | Lys | Pro | Arg | Arg | |
| | | | | 180 | | | | | 185 | | | | 190 | | |

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1094 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 3..624
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | | | |
|---|-----|----|----|
| CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG | 47 | | |
| Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln | | | |
| 1 | 5 | 10 | 15 |
| CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC | 95 | | |
| Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His | | | |
| 20 | 25 | 30 | |
| CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC | 143 | | |
| Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys | | | |
| 35 | 40 | 45 | |

- 40 -

| | |
|---|------|
| CAG CCC CGG GAG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr 50 55 60 | 191 |
| GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly 65 70 75 | 239 |
| GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His 80 85 90 95 | 287 |
| CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu 100 105 110 | 335 |
| GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys 115 120 125 | 383 |
| AAA AAG GAC AGT GCT GTG AAG CCA GAC AGG GCT GCC ACT CCC CAC CAC Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His 130 135 140 | 431 |
| CGT CCC CAG CCC CGT TCT GTT CCG GGC TGG GAC TCT GCC CCC GGA GCA Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala 145 150 155 | 479 |
| CCC TCC CCA GCT GAC ATC ACC CAT CCC ACT CCA GCC CCA GGC CCC TCT Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser 160 165 170 175 | 527 |
| GCC CAC GCT GCA CCC AGC ACC ACC AGC GCC CTG ACC CCC GGA CCT GCC Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala 180 185 190 | 575 |
| GCT GCC GCT GCC GAC GCA GCT TCC TCC GTT GCC AAG GGC GGG GCT T Ala Ala Ala Ala Asp Ala Ala Ser Ser Val Ala Lys Gly Gly Ala 195 200 205 | 624 |
| AGAGCTAAC CCAGACACCT GCAGGTGCCG GAAGCTGCGA AGGTGACACA TGGCTTTCA | 684 |
| GAATCAGCAG GGTGACTTGC CTCAGAGGCT ATATCCCAGT GGGGAACAA AGGGGAGCCT | 744 |
| GGTAAAAAAC AGCCAAGCCC CCAAGACCTC AGCCCAGGCA GAAGCTGCTC TAGGACCTGG | 804 |
| GCCTCTCAGA GGGCTTTCT GCCATCCCTT GTCTCCCTGA GGCCATCATC AAACAGGACA | 864 |
| GAGTTGGAAG AGGAGACTGG GAGGCAGCAA GAGGGGTAC ATACCAGCTC AGGGGAGAAT | 924 |
| GGAGTACTGT CTCAGTTCT AACCACTCTG TGCAAGTAAG CATCTTACAA CTGGCTCTTC | 984 |
| CTCCCCCTCAC TAAGAAGACC CAAACCTCTG CATAATGGGA TTTGGGCTTT GGTACAAGAA | 1044 |
| CTGTGACCCC CAACCCTGAT AAAAGAGATG GAAGGAAAAA AAAAAAAA | 1094 |

- 41 -

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65 70 75 80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
85 90 95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
100 105 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
115 120 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
130 135 140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
145 150 155 160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala
165 170 175

His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala
180 185 190

Ala Ala Ala Asp Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
195 200 205

- 42 -

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | |
|---|-----|
| CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG | 47 |
| Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln | |
| 1 5 10 15 | |
| CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC | 95 |
| Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His | |
| 20 25 30 | |
| CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC | 143 |
| Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys | |
| 35 40 45 | |
| CAG CCC CGG GAG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC | 191 |
| Gln Pro Arg Glu Val Val Pro Leu Thr Val Glu Leu Met Gly Thr | |
| 50 55 60 | |
| GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT | 239 |
| Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly | |
| 65 70 75 | |
| GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC | 287 |
| Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His | |
| 80 85 90 95 | |
| CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG | 335 |
| Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu | |
| 100 105 110 | |
| GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA | 383 |
| Gly Glu Met Ser Leu Glu His Ser Gln Cys Glu Cys Arg Pro Lys | |
| 115 120 125 | |
| AAA AAG GAC AGT GCT GTG AAG CCA GAT AGC CCC AGG CCC CTC TGC CCA | 431 |
| Lys Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro | |
| 130 135 140 | |

- 43 -

| | |
|---|-----|
| CGC TGC ACC CAG CAC CAG CGC CCT GAC CCC CGG ACC TGC CGC TGC Arg Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys 145 150 155 | 479 |
| CGC TGC CGA CGC CGC AGC TTC CTC CGT TGC CAA GGG CGG GGC TTA GAG Arg Cys Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu 160 165 170 175 | 527 |
| CTC AAC CCA GAC ACC TGC AGG TGC CGG AAG CTG CGA AGG TGACACATGG Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg 180 185 | 576 |
| CTTTTCAGAC TCAGCAGGGT GACTTGCTC AGAGGCTATA TCCCAGTGGG GGAACAAAGG | 636 |
| GGAGCCTGGT AAAAAACAGC CAAGCCCCA AGACCTCAGC CCAGGCAGAA GCTGCTCTAG | 696 |
| GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC CATCATCAAA | 756 |
| CAGGACAGAG TTGGAAGAGG AGACTGGAG GCAGCAAGAG GGGTCACATA CCAGCTCAGG | 816 |
| GGAGAACATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT CTTACAAC | 876 |
| GCTCTTCCTC CCCTCACTAA GAAGACCAA ACCTCTGCAT AATGGGATTG GGGCTTTGGT | 936 |
| ACAAGAACCTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAAAA AAAAAAAA | 993 |

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Pro | Leu | Leu | Arg | Arg | Leu | Leu | Leu | Ala | Ala | Leu | Leu | Gln | Leu |
| 1 | | | | | 5 | | | | | 10 | | | | 15 | |
| Ala | Pro | Ala | Gln | Ala | Pro | Val | Ser | Gln | Pro | Asp | Ala | Pro | Gly | His | Gln |
| | | | | | 20 | | | | 25 | | | | 30 | | |
| Arg | Lys | Val | Val | Ser | Trp | Ile | Asp | Val | Tyr | Thr | Arg | Ala | Thr | Cys | Gln |
| | | | | | 35 | | | 40 | | | | 45 | | | |
| Pro | Arg | Glu | Val | Val | Val | Pro | Leu | Thr | Val | Glu | Leu | Met | Gly | Thr | Val |
| | | | | | 50 | | | 55 | | | 60 | | | | |
| Ala | Lys | Gln | Leu | Val | Pro | Ser | Cys | Val | Thr | Val | Gln | Arg | Cys | Gly | Gly |
| | | | | | 65 | | | 70 | | | 75 | | | 80 | |

- 44 -

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Cys | Pro | Asp | Asp | Gly | Leu | Glu | Cys | Val | Pro | Thr | Gly | Gln | His | Gln |
| | | | | | | 85 | | | | 90 | | | | 95 | |
| Val | Arg | Met | Gln | Ile | Leu | Met | Ile | Arg | Tyr | Pro | Ser | Ser | Gln | Leu | Gly |
| | | | | | | 100 | | | 105 | | | | 110 | | |
| Glu | Met | Ser | Leu | Glu | Glu | His | Ser | Gln | Cys | Glu | Cys | Arg | Pro | Lys | Lys |
| | | | | | | 115 | | | 120 | | | | 125 | | |
| Lys | Asp | Ser | Ala | Val | Lys | Pro | Asp | Ser | Pro | Arg | Pro | Leu | Cys | Pro | Arg |
| | | | | | | 130 | | | 135 | | | 140 | | | |
| Cys | Thr | Gln | His | His | Gln | Arg | Pro | Asp | Pro | Arg | Thr | Cys | Arg | Cys | Arg |
| | | | | | | 145 | | | 150 | | | 155 | | 160 | |
| Cys | Arg | Arg | Arg | Ser | Phe | Leu | Arg | Cys | Gln | Gly | Arg | Gly | Leu | Glu | Leu |
| | | | | | | 165 | | | 170 | | | 175 | | | |
| Asn | Pro | Asp | Thr | Cys | Arg | Cys | Arg | Lys | Leu | Arg | Arg | | | | |
| | | | | | | 180 | | | 185 | | | | | | |

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 858 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..431

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CC | ATG | AGC | CCT | CTG | CTC | CGC | CGC | CTG | CTG | CTC | GCC | GCA | CTC | CTG | CAG | 47 |
| Met | Ser | Pro | Leu | Leu | Arg | Arg | Leu | Leu | Leu | Ala | Ala | Ala | Leu | Leu | Gln | |
| 1 | | | 5 | | | | | 10 | | | | | | 15 | | |
| CTG | GCC | CCC | GCC | CAG | GCC | CCT | GTC | TCC | CAG | CCT | GAT | GCC | CCT | GGC | CAC | 95 |
| Leu | Ala | Pro | Ala | Gln | Ala | Pro | Val | Ser | Gln | Pro | Asp | Ala | Pro | Gly | His | |
| 20 | | | 25 | | | | | | | | | | | 30 | | |
| CAG | AGG | AAA | GTG | GTG | TCA | TGG | ATA | GAT | GTG | TAT | ACT | CGC | GCT | ACC | TGC | 143 |
| Gln | Arg | Lys | Val | Val | Ser | Trp | Ile | Asp | Val | Tyr | Thr | Arg | Ala | Thr | Cys | |
| 35 | | | 40 | | | | | | | | | | | 45 | | |
| CAG | CCC | CGG | GAG | GTG | GTG | GTG | CCC | TTG | ACT | GTG | GAG | CTC | ATG | GGC | ACC | 191 |
| Gln | Pro | Arg | Glu | Val | Val | Val | Pro | Leu | Thr | Val | Glu | Leu | Met | Gly | Thr | |
| 50 | | | 55 | | | | | | | | | | | 60 | | |

- 45 -

- 46 -

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65 70 75 80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
85 90 95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
100 105 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
115 120 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg
130 135 140

- 47 -

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 910 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..305

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

| | |
|---|-----|
| CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG | 47 |
| Met Ser Pro Leu Leu Arg Arg Leu Leu Ala Ala Leu Leu Gln | |
| 1 5 10 15 | |
| CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC | 95 |
| Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His | |
| 20 25 30 | |
| CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC | 143 |
| Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys | |
| 35 40 45 | |
| CAG CCC CGG GAG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC | 191 |
| Gln Pro Arg Glu Val Val Pro Leu Thr Val Glu Leu Met Gly Thr | |
| 50 55 60 | |
| G TG GGC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT | 239 |
| Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly | |
| 65 70 75 | |
| GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC | 287 |
| Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His | |
| 80 85 90 95 | |
| CAA GTC CGG ATG CAG ACC TAAAAAAAAG GACAGTGCTG TGAAGCCAGA | 335 |
| Gln Val Arg Met Gln Thr | |
| 100 | |
| CAGGGCTGCC ACTCCCCACC ACCGTCCCCA GCCCGTTCT GTTCCGGGCT GGGACTCTGC | 395 |
| CCCCGGAGCA CCCTCCCCAG CTGACATCAC CCATCCCACT CCAGCCCCAG GCCCCTCTGC | 455 |
| CCACGCTGCA CCCAGCACCA CCAGCGCCCT GACCCCCGGA CCTGCCGCTG CCGCTGCCGA | 515 |
| CGCCGCAGCT TCCTCCGTTG CCAAGGGCGG GGCTTAGAGC TCAACCCAGA CACCTGCAGG | 575 |
| TGCCGGAAGC TGCAGAAGGTG ACACATGGCT TTTCAGACTC AGCAGGGTGA CTTGCCTCAG | 635 |
| AGGCTATATC CCAGTGGGGA ACAAAAGAGGA GCCTGGTAAA AAACAGCCAA GCCCCCCAAGA | 695 |

- 48 -

| | |
|--|-----|
| CCTCAGCCCA GGCAGAACGCT GCTCTAGGAC CTGGGCCTCT CAGAGGGCTC TTCTGCCATC | 755 |
| CCTTGTCTCC CTGAGGCCAT CATCAAACAG GACAGAGTTG GAAGAGGAGA CTGGGAGGCA | 815 |
| GCAAGAGGGG TCACATACCA GCTCAGGGGA GAATGGAGTA CTGTCTCAGT TTCTAACAC | 875 |
| TCTGTGCAAG TAAGCATCTT ACAACTGGCT CTTCC | 910 |

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

| | | | |
|---|----|----|----|
| Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu | | | |
| 1 | 5 | 10 | 15 |
| Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln | | | |
| 20 | 25 | 30 | |
| Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln | | | |
| 35 | 40 | 45 | |
| Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val | | | |
| 50 | 55 | 60 | |
| Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly | | | |
| 65 | 70 | 75 | 80 |
| Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln | | | |
| 85 | 90 | 95 | |
| Val Arg Met Gln Thr | | | |
| 100 | | | |

- 49 -

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCACCACCT CCCTGGGCTG GCATGTGGCA CGTGCATAAA CG

42

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGTTGTTTGA CCACATTGCC CATGAGTTCC ATGCTCAGAG GC

42

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCCTGGGG CTGGAGTGGG ATGGATGATG TCAGCTGG

38

- 50 -

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGGGCAGAG GATCCTGGGG CTGTCTGCC TCACAGCACT

40

CLAIMS:

1. A biologically isolated proteinaceous molecule having the following characteristics:
 - (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
2. A proteinaceous molecule according to claim 1 wherein the molecule exhibits at least one of the following properties:
 - (i) an ability to induce vascular endothelial cells;
 - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
 - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
3. A proteinaceous molecule according to claim 1 or 2 wherein said molecule has the capacity to induce astroglial proliferation.
4. A proteinaceous molecule according to claim 1 wherein said molecule is of human origin.
5. A proteinaceous molecule according to claim 1 wherein said molecule is of non-human origin.
6. A proteinaceous molecule according to claim 5 wherein said molecule is of livestock animal, companion animal, laboratory test animal, avian, fish or reptilian origin.
7. A proteinaceous molecule according to claim 5 wherein said molecule is encoded by a gene located at chromosome 11q13.

- 52 -

8. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
9. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 40%.
10. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 60-70%.
11. A proteinaceous molecule according to claim 1 comprising a sequence of amino acids as set forth in SEQ ID NO:4 or a part, fragment, derivative or analogue thereof.
12. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:6 or a part, fragment, derivative or analogue thereof.
13. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:8 or a part, fragment, derivative or analogue thereof.
14. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:10 or a part, fragment, derivative or analogue thereof.
15. A recombinant molecule having the following characteristics:
 - (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.

16. A recombinant molecule having the following characteristics:
 - (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.
17. A recombinant molecule having the following characteristics:
 - (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.
18. A recombinant molecule having the following characteristics:
 - (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.
19. A recombinant molecule according to claim 15 or 16 or 17 or 18 having at least one of the following properties:
 - (a) an ability to induce vascular endothelial cells;
 - (b) an ability to interact with *flt1/flki* family of receptors;
 - (c) an ability to induce cell migration, cell survival and/or increase intracellular levels of alkaline phosphatase.
20. A recombinant molecule according to claim 15 or 16 or 17 or 18 having the capacity to induce astroglial proliferation.

21. A recombinant molecule according to claim 20 wherein the molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6.

22. A peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a derivative or chemical equivalent thereof.

23. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:6 or a chemical equivalent thereof.

24. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:8 or a chemical equivalent thereof.

25. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:10 or a chemical equivalent thereof.

26. A nucleic acid molecule comprising a sequence of nucleotides or complementary to a sequence encoding a proteinaceous molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).

27. A nucleic acid molecule according to claim 26 wherein the proteinaceous molecule exhibits at least one of the following properties:

- (i) an ability to induce vascular endothelial cells;
- (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
- (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

28. A nucleic acid molecule according to claim 27 wherein the proteinaceous molecule has the capacity to induce astroglial proliferation.

- 55 -

29. A nucleic acid molecule according to claim 28 wherein said molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:6.

30. A nucleic acid molecule according to claim 1 wherein said molecule is of human origin.

31. A nucleic acid molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.

32. A nucleic acid molecule according to claim 26 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.

33. A nucleic acid molecule according to claim 26 encoding a murine homologue of human VEGF and comprising a nucleotide sequence substantially as set forth in Figure 9.

34. A pharmaceutical composition comprising a proteinaceous molecule according to claim 1 or 2 or 3 or 11 and one or more pharmaceutically acceptable carriers and/or diluents.

35. A method for preparing a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said method comprising expressing a nucleic acid molecule encoding said recombinant

- 56 -

molecule by a suitable host grown under conditions effective to synthesise said recombinant molecule and then isolating said molecule.

36. A method according to claim 35 wherein the nucleic acid molecule comprises a sequence of nucleotides as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or is capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.

37. A method of inducing astrogial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astrogial proliferation.

38. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

39. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

40. A method of promoting neuronal survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 57 -

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

ABSTRACT

The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

| | |
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| APPROVED BY | O.G. FIG. CLASS SUBCLASS |
| DRAFTSMAN | |

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1/52

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| 2/52 | 3/52 |
| <i>Fig.1(i)</i> | <i>Fig.1(ii)</i> |
| 4/52 | 5/52 |
| <i>Fig.1(iii)</i> | <i>Fig.1(iv)</i> |

| | |
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| APPROVED BY DRAFTSMAN | O.G. FIG. CLASS 960 CLASS |
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2/52

| | |
|-----|---|
| 1 | TCGGCCTCC GAAACC ATG AAC TTT CTG Met Asn Phe Leu 1 |
| 50 | CTT GCC TTG CTG CTC TAC CTC CAC Leu Ala Leu Leu Leu Tyr Leu His 15 |
| 98 | CCC ATG GCA GAA GGA GGA GGG CAG Pro Met Ala Glu Gly Gly Gly Gln 30 35 |
| 146 | ATG GAT GTC TAT CAG CGC AGC TAC Met Asp Val Tyr Gln Arg Ser Tyr 45 50 |
| 194 | GAC ATC TTC CAG GAG TAC CCT GAT Asp Ile Phe Gln Glu Tyr Pro Asp 60 65 |
| 242 | TCC TGT GTG CCC CTG ATG CGA TGC Ser Cys Val Pro Leu Met Arg Cys 80 |
| 290 | CTC GAG TGT GTG CCC ACT GAG GAG Leu Glu Cys Val Pro Thr Glu Glu 95 |
| 338 | CGG ATC AAA CCT CAC CAA GGC CAG Arg Ily Lys Pro His Gln Gly Gln 110 115 |

Fig.1(i)

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. CLASS & SUBCLASS |
| DRAFTSMAN | |

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| | |
|---------------------------------|-----|
| CTG TCT TGG GTG CAT TGG AGC | 49 |
| Leu Ser Trp Val His Trp Ser | |
| 5 | 10 |
| CAT GCC AAG TGG TCC CAG GCT GCA | 97 |
| His Ala Lys Trp Ser Gln Ala Ala | |
| 20 | 25 |
| AAT CAT CAC GAA GTG GTG AAG TTC | 145 |
| Asn His His Glu Val Val Lys Phe | |
| 40 | |
| TGC CAT CCA ATC GAG ACC CTG GTG | 193 |
| Cys His Pro Ile Glu Thr Leu Val | |
| 55 | |
| GAG ATC GAG TAC ATC TTC AAG CCA | 241 |
| Glu Ile Glu Tyr Ile Phe Lys Pro | |
| 70 | 75 |
| GGG GGC TGC TGC AAT GAC GAG GGC | 289 |
| Gly Gly Cys Cys Asn Asp Glu Gly | |
| 85 | 90 |
| TCC AAC ATC ACC ATG CAG ATT ATG | 337 |
| Ser Asn Ile Thr Met Gln Ile Met | |
| 100 | 105 |
| CAC ATA GGA GAG ATG AGC TTC CTA | 385 |
| His Ile Gly Glu Met Ser Phe Leu | |
| 120 | |

Fig.1(ii)

| | |
|----------------|--------------------|
| APPROVED BY | O.G. FIG. CLASS |
| DRAFTSMAN | CLASS SUBCLASS |

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4/52

| | |
|-----|--|
| 386 | CAG CAC AAC AAA TGT GAA TGC AGA Gln His Asn Lys Cys Glu Cys Arg 125 130 |
| 434 | GAA AAT CCC TGT GGG CCT TGC TCA Glu Asn Pro Cys Gly Pro Cys Ser 140 145 |
| 482 | CAA GAT CCG CAG ACG TGT AAA TGT Gln Asp Pro Gln Thr Cys Lys Cys 160 |
| 530 | TGC AAG GCG AGG CAG CTT GAG TTA Cys Lys Ala Arg Gln Leu Glu Leu 175 |
| 578 | AAG CCG AGG CGG TGAGCCGGGC AGGAG Lys Pro Arg Arg 190 |
| 630 | GAACCAGATC TCTCACCAAGG |

Fig. 1(iii)

| | |
|----------------|-----------------------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CLASS SUBCLASS W 58/2700 |

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Q/AU960094

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| | |
|---------------------------------|-----|
| CCA AAG AAA GAT AGA GCA AGA CAA | 433 |
| Pro Lys Lys Asp Arg Ala Arg Gln | |
| 135 | |
| GAG CGG AGA AAG CAT TTG TTT GTA | 481 |
| Glu Arg Arg Lys His Leu Phe Val | |
| 150 | 155 |
| TCC TGC AAA AAC ACA GAC TCG CGT | 529 |
| Ser Cys Lys Asn Thr Asp Ser Arg | |
| 165 | 170 |
| AAC GAA CGT ACT TGC AGA TGT GAC | 577 |
| Asn Glu Arg Thr Cys Arg Cys Asp | |
| 180 | 185 |
| GAAGG AGCCTCCCTC AGCGTTTCGG | 629 |

649

Fig.1(iv)

| | |
|----------------|----------------------|
| APPROVED BY | QC FIG W0307/0007 |
| DRAFTSMAN | CLASS SUBCLASS |

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6/52

| | |
|-------------------|-------------------|
| 7/52 | 8/52 |
| <i>Fig. 2(i)</i> | <i>Fig. 2(ii)</i> |
| 9/52 | 10/52 |
| <i>Fig 2(iii)</i> | <i>Fig 2(iv)</i> |
| 11/52 | 12/52 |
| <i>Fig 2(v)</i> | <i>Fig 2(vi)</i> |

| | |
|----------------|---------------------|
| APPROVED BY | G.W. FG 96/0007 |
| DRAFTSMAN | CLASS SUBCLASS |

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| | |
|-----|--|
| 1 | CC ATG AGC CCT CTG CTC CGC CGC Met Ser Pro Leu Leu Arg Arg 1 5 |
| 48 | CTG GCC CCC GCC CAG GCC CCT GTC Leu Ala Pro Ala Gln Ala Pro Val 20 |
| 96 | CAG AGG AAA GTG GTG TCA TGG ATA Gln Arg Lys Val Val Ser Trp Ile 35 |
| 144 | CAG CCC CGG GAG GTG GTG GTG CCC Gln Pro Arg Glu Val Val Val Pro 50 55 |
| 192 | GTG GCC AAA CAG CTG GTG CCC AGC Val Ala Lys Gln Leu Val Pro Ser 65 70 |
| 240 | GGC TGC TGC CCT GAC GAT GGC CTG Gly Cys Cys Pro Asp Asp Gly Leu 80 85 |
| 288 | CAA GTC CGG ATG CAG ATC CTC ATG Gln Val Arg Met Gln Ile Leu Met 100 |
| 336 | GGG GAG ATG TCC CTG GAA GAA CAC Gly Glu Met Ser Leu Glu Glu His 115 |

Fig.2(i)

| | | | |
|----------------|-----------------------|-------|----------|
| APPROVED BY | O.G. FIG DRAFTSMAN | CLASS | SUBCLASS |
|----------------|-----------------------|-------|----------|

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| | |
|---|-----|
| CTG CTG CTC GCC GCA CTC CTG CAG Leu Leu Leu Ala Ala Leu Leu Gln 10 15 | 47 |
| TCC CAG CCT GAT GCC CCT GGC CAC Ser Gln Pro Asp Ala Pro Gly His 25 30 | 95 |
| GAT GTG TAT ACT CGC GCT ACC TGC Asp Val Tyr Thr Arg Ala Thr Cys 40 45 | 143 |
| TTG ACT GTG GAG CTC ATG GGC ACC Leu Thr Val Glu Leu Met Gly Thr 60 | 191 |
| TGC GTG ACT GTG CAG CGC TGT GGT Cys Val Thr Val Gln Arg Cys Gly 75 | 239 |
| GAG TGT GTG CCC ACT GGG CAG CAC Glu Cys Val Pro Thr Gly Gln His 90 95 | 287 |
| ATC CGG TAC CCG AGC AGT CAG CTG Ile Arg Tyr Pro Ser Ser Gln Leu 105 110 | 335 |
| AGC CAG TGT GAA TGC AGA CCT AAA Ser Gln Cys Glu Cys Arg Pro Lys 120 125 | 383 |

Fig. 2(ii)

| | |
|-----------------------------|-----------------------------|
| APPROVED BY DRAFTSMAN | O.G. FIG. CLASS 6100 ASS |
|-----------------------------|-----------------------------|

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| | |
|-----|---|
| 384 | AAA AAG GAC AGT GCT GTG AAG CCA Lys Lys Asp Ser Ala Val Lys Pro 130 135 |
| 432 | CGT CCC CAG CCC CGT TCT GTT CCG Arg Pro Gln Pro Arg Ser Val Pro 145 150 |
| 480 | CCC TCC CCA GCT GAC ATC ACC CAT Pro Ser Pro Ala Asp Ile Thr His 160 165 |
| 528 | GCC CAC GCT GCA CCC AGC ACC ACC Ala His Ala Ala Pro Ser Thr Thr 180 |
| 576 | GCT GCC GCT GCC GAC GCC GCA GCT Ala Ala Ala Ala Asp Ala Ala Ala 195 |

Fig. 2(iii)

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. CLASS & SUBCLASS |
| DRAFTSMAN | |

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| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GAC | AGG | GCT | GCC | ACT | CCC | CAC | CAC | 431 | |
| Asp | Arg | Ala | Ala | Thr | Pro | His | His | | |
| | | | | | | | | 140 | |
| GGC | TGG | GAC | TCT | GCC | CCC | GGA | GCA | 479 | |
| Gly | Trp | Asp | Ser | Ala | Pro | Gly | Ala | | |
| | | | | | | | | 155 | |
| CCC | ACT | CCA | GCC | CCA | GGC | CCC | TCT | 527 | |
| Pro | Thr | Pro | Ala | Pro | Gly | Pro | Ser | | |
| | | | | | | | | 170 | |
| | | | | | | | | 175 | |
| AGC | GCC | CTG | ACC | CCC | GGA | CCT | GCC | 575 | |
| Ser | Ala | Leu | Thr | Pro | Gly | Pro | Ala | | |
| | | | | | | | | 185 | |
| | | | | | | | | 190 | |
| TCC | TCC | GTT | GCC | AAG | GGC | GGG | GCT | T | 624 |
| Ser | Ser | Val | Ala | Lys | Gly | Gly | Gly | Ala | |
| | | | | | | | | 200 | |
| | | | | | | | | 205 | |

Fig.2(iv)

| | | | |
|----------------|------------------------|-------|----------|
| APPROVED BY | O.G. FIG. 1996/0094 | CLASS | SUBCLASS |
| DRAFTSMAN | | | |

08/76588
1996/0094

11/52

| | | | | |
|------|------------|------------|------------|--|
| 625 | AGAGCTCAAC | CCAGACACCT | GCAGGTGCCG | |
| 685 | GACTCAGCAG | GGTGACTTGC | CTCAGAGGCT | |
| 745 | GGTAAAAAAC | AGCCAAGCCC | CCAAGACCTC | |
| 805 | GCCTCTCAGA | GGGCTTTCT | GCCATCCCTT | |
| 865 | GAGTTGGAAG | AGGAGACTGG | GAGGCAGCAA | |
| 825 | GGAGTACTGT | CTCAGTTCT | AACCCTCTG | |
| 985 | CTCCCCTCAC | TAAGAAGACC | CAAACCTCTG | |
| 1045 | CTGTGACCCC | CAACCCTGAT | AAAAGAGATG | |

Fig. 2(v)

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. CLASS / SUBCLASS |
| DRAFTSMAN | |

08/765588
PCT/AU96/00094

12/52

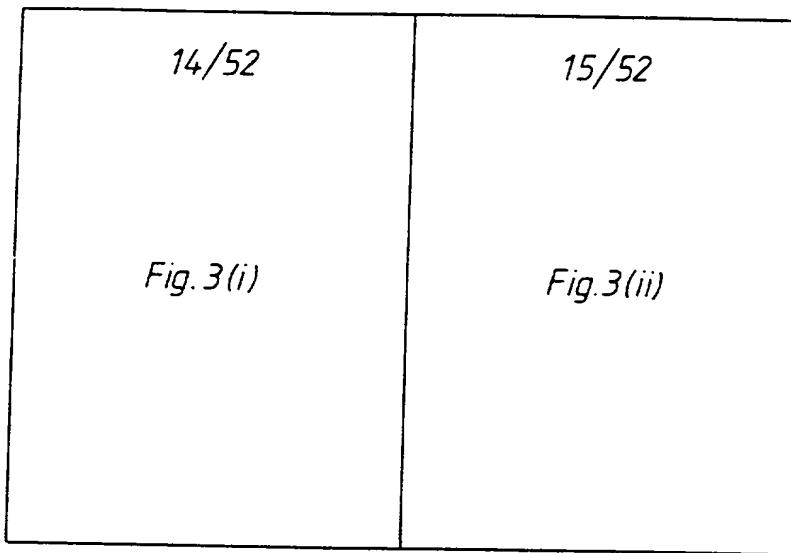
| | | | |
|-------------|-------------|------------|------|
| GAAGCTGCGA | AGGTGACACA | TGGCTTTCA | 684 |
| ATATCCCAGT | GGGGGAACAA | AGGGGAGCCT | 744 |
| AGCCCAGGCA | GAAGCTGCTC | TAGGACCTGG | 804 |
| GTCTCCCTGA | GGCCATCATC | AAACAGGACA | 864 |
| GAGGGGTCAC | ATACCAGCTC | AGGGGAGAAT | 924 |
| TGCAAGTAAG | CATCTTACAA | CTGGCTCTTC | 984 |
| CATAATGGGA | TTTGGGCTTT | GGTACAAGAA | 1044 |
| GAAGGAAAAAA | AAAAAAAAAAA | | 1094 |

Fig. 2(vi)

| | |
|-----------|-------------------|
| APPROVED | O.G. FIG. |
| BY | CLASS 96 94 GLASS |
| DRAFTSMAN | |

08/765588
PCT/AU9600094

13/52



| | |
|-----------|---------------------|
| APPROVED | Q656 |
| BY | Q656/0007 |
| DRAFTSMAN | CLASS SUBCLASS |

086765588
Q656/0004

14/52

>VEGF_HUMAN VEGF_HUMAN VASCULAR ENDOTHELIAL
(VASCULAR 215 AA.
LENGTH = 215

SCORE = 181 (92.4 BITS), EXPECT = 6.4e-20,
IDENTITIES = 33/75 (44%), POSITIVES = 48/75

QUERY: 31 HQRKVVSVIDVYTRATCQPREVVVPLTVEL
+++ VV +DVY R+ C+P E +V + E

SBJCT: 36 NHHEVVVKFMDVYQRSYCHPIETLVDIFQEY

QUERY: 91 PTGQHQVRMQILMIR 105
PT + + MQI+ I+

SBJCT: 96 PTEESNITMQIMRIK 110

SCORE = 76 (38.8 BITS), EXPECT = 0.0011,
IDENTITIES = 12/19 (63%), POSITIVES = 16/19

QUERY: 110 QLGEMSLEEHSQCECRPKK 128
++GEMS +H+ CECRPKK

SBJCT: 116 HIGEMSFLQHNKCECRPKK 134

SCORE = 72 (36.8 BITS), EXPECT = 0.0046,
IDENTITIES = 14/21 (66%), POSITIVES = 15/21

QUERY: 202 RCQGRGLELNPDTCRCKLRR 222
RC +R LEQN TCRC K RR

SBJCT: 195 RCKARQLELNERTCRCDKPRR 215

SCORE = 46 (23.5 BITS), EXPECT = 47.,
IDENTITIES = 6/10 (60%), POSITIVES = 9/10

QUERY: 187 DPRTCRCKR 196
DP+TC+C C+

SBJCT: 181 DPQTCKCSCK 190
SUBSTITUTE SHEET (RULE 26)

Fig.3(i)

| | |
|----------------|--------------------------------|
| APPROVED BY | O.G. FIG. MOT 2000 APR 2007 |
| DRAFTSMAN | CLASS SUBCLASS |

08/765588
PCTAU96/00094

15/52

GROWTH FACTOR PRECURSOR (VEGF)

$P = 6.4e-20$
(64%)

MGTVAKQLVPSCVTVQRCGGCCPDDGLECV 90
 + PSCV + RCGGCC D+GLECV
 PDEIEYIFKPSCVPLMRCGGCCNDEGLECV 95

POISSON $P(2) = 9.1e-12$
(84%)

POISSON $P(3) = 3.6e-18$
(71%)

POISSON $P(4) = 7.3e-10$
(90%)

Fig. 3(i)

| | | | |
|----------------|-------------------------|-------|----------|
| APPROVED BY | Q.G.FIG. WAS/97/0007 | CLASS | SUBCLASS |
| DRAFTSMAN | | | |

08/765588
PCU/00094

16/52

| | |
|--------------------|-------------------|
| 17/52 | 18/52 |
| <i>Fig. 4(i)</i> | <i>Fig. 4(ii)</i> |
| 19/52 | 20/52 |
| <i>Fig. 4(iii)</i> | <i>Fig. 4(iv)</i> |

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SUBCLASS |
| DRAFTSMAN | |

08/765588
PCP/AU96/00094

17/52

Gap Weight:3.00 Average Match:1.000
Length Weight:0.100 Average Mismatch:-0.900
Quality:100.9 Length:739
Ratio:0.175 Gaps:30
Percent Percent
Similarity:69.703 Identity:69.703

Fig. 4(i)

| | |
|-----------------------------|--------------------------------|
| APPROVED BY DRAFTSMAN | O.G. FIG. CLASS 512/200 ASS |
|-----------------------------|--------------------------------|

08/765588
PETALU 96/00094

18/52

| | | |
|-------------------------------|----|-----|
| TGCTCGCCGGCACT..... | CC | 67 |
| | | |
| ...TGGGTGCATTGGAGCCTTGCCT | | 56 |
| TGTCTCCCAGCCTGATGCCCTGGC | | 117 |
| | | |
| GTGGTCCCAGGCTGCA.CCCATGGC | | 105 |
| .AAGTGGTG....TCATGGATAGAT | | 147 |
| | | |
| GAAGTGGTGAAGTTCATG....GAT | | 151 |
| CCCCGGGAG...GTGGTGGTGCCCT | | 193 |
| | | |
| CCAATCGAGACCCTGGTGGACATCT | | 200 |
| GGGCACCGTGGCCAAACAGCTGGTG | | 234 |
| | | |
| GTACATCTT...CAA.....G | | 238 |
| GTGGTGGCTGCTGCCCTGACGATGG | | 284 |
| | | |
| GCGGGGGCTGCTGCAATGACGAGGG | | 288 |
| CACCAAGTCCGGATGCAGAT..... | | 329 |
| | | |
| TCCAACATCACCATGCAGATTATGC | | 338 |

Fig.4(ii)
SUBSTITUTE SHEET (RULE 26)

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SUBCLASS |
| DRAFTSMAN | W0 627007 |

08/765588
PCT/AU9600094

19/52

330 CCTCATGATCCGGTAC
339 GGATCAAACCTCA.....C

369 GTCCCTGGAAGAACACAGCCAGTGT
376 GAGCTTCCTACAGCACAAACAAATGT

419 GTGCTGTGAAGCCAGACAGGGCTGC
423 G.....AGCAAGACAAG.....

469 CGTTCTGTTCCGGGCTGGGACTCTG
443 ...TGTGGGCCTTGCTCAGA.....

519 CATCACCCATCCCACCTCCAGCCCCA

468

569 GC.....ACCACCAGCGCCC
469 GCATTGTTGTACAA.....

609 TGCCGACGCCGCAGCTCCTCCGTT
509 TG.CAAAAAACACAGACTC..GCGTT

657 AACCCAGACACCTGCAGGTGCCGGA
554 AACGAAACGTACTTGCAGATGTGACA

Fig. 4(iii)

| | |
|-----------------------------|--------------------|
| APPROVED BY DRAFTSMAN | O.G. FIG. CLASS |
| | CLASS |

08/765588
PCTAU96/00094

20/52

| | |
|-------------------------------|-----|
| CGAGCAGTCAGC . . . TGGGGGAGAT | 368 |
| | |
| CAAG . . GCCAGCACATAGGAGAGAT | 375 |
| GAATGCAGACCTAAAAAAAAGGACA | 418 |
| | |
| GAATGCAGACC . . . AAAGAAAGATA | 422 |
| CACTCCCCACCACCGTCCCCAGCCC | 468 |
| | |
| AAAATCCC | 442 |
| CCCCCGGAGCACCCCTCCCCAGCTGA | 518 |
| | |
| ... GCGGAGAA | 467 |
| GGCCCCTCTGCCAACGCTGCACCCA | 568 |
| | |
| A | 468 |
| TGACCCCCGGACCTGCCGCTGCCGC | 608 |
| | |
| . GATCCCGAGACGTGTAAATGTTCC | 508 |
| GCCAAGGGCGGGGC . . TTAGAGCTC | 656 |
| | |
| GC . . AAGGCGAGGCAGCTTGAGTTA | 553 |
| AGCTGCGAAGGTGA | 695 |
| AGCCGAGGCAGGTGA | 592 |

Fig.4(iv)

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. CLASS / SUBCLASS |
| DRAFTSMAN | |

08/765588
PCPAU96/00094

21/52

| | | |
|------------------|------------------|-------------------|
| 22/52 | 23/52 | 24/52 |
| <i>Fig.5(i)</i> | <i>Fig.5(ii)</i> | <i>Fig.5(iii)</i> |
| 25/52 | 26/52 | 27/52 |
| <i>Fig.5(iv)</i> | <i>Fig.5(v)</i> | <i>Fig.5(vi)</i> |

| | |
|----------------|-----------------------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CLASS SUBCLASS W096/2700 |

08/765588
TQ/AU9600094

22/52

165SOMSQ.MSF.msf MSF:687

Type: D Tuesday, June 20, 1995

Check: 3140

1

| | |
|-------------|--------------------------|
| VEGF165 | ATGAACCTTCTGCTGTCTGGGTG |
| SOM175 | ATGAGCCCTCTGCTCCGCCGCCTG |
| SOM175-e6 | ATGAGCCCTCTGCTCCGCCGCCTG |
| SOM175-e6&7 | ATGAGCCCTCTGCTCCGCCGCCTG |
| SOM175-e4 | ATGAGCCCTCTGCTCCGCCGCCTG |

81

| | |
|-------------|--------------------------|
| VEGF165 | CACCCATGGCAGAAGGAGGAGGGC |
| SOM175 | TGCCCCTGGCCACCAGAGGAAAGT |
| SOM175-e6 | TGCCCCTGGCCACCAGAGGAAAGT |
| SOM175-e6&7 | TGCCCCTGGCCACCAGAGGAAAGT |
| SOM175-e4 | TGCCCCTGGCCACCAGAGGAAAGT |

161

| | |
|-------------|--------------------------|
| VEGF165 | CCAATCGAGACCCTGGTGGACATC |
| SOM175 | GTGGTGGTGCCCTTGACTG.TGGA |
| SOM175-e6 | GTGGTGGTGCCCTTGACTG.TGGA |
| SOM175-e6&7 | GTGGTGGTGCCCTTGACTG.TGGA |
| SOM175-e4 | GTGGTGGTGCCCTTGACTG.TGGA |

241

| | |
|-------------|-------------------------|
| VEGF165 | GATGCGATGCGGGGCTGCTGCAA |
| SOM175 | GCAGCGCTGTGGTGGCTGCTGCC |
| SOM175-e6 | GCAGCGCTGTGGTGGCTGCTGCC |
| SOM175-e6&7 | GCAGCGCTGTGGTGGCTGCTGCC |
| SOM175-e4 | GCAGCGCTGTGGTGGCTGCTGCC |

Fig.5(i)

| | |
|-----------------------------|---|
| APPROVED BY DRAFTSMAN | O.G. FIG. CL 100 SUBCLASS |
|-----------------------------|---|

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PCTAU96/00094

23/52

CATTGGAGCCTTGCCTGCTGCTCTACC
 CTGCTCGCCGCACTCCTGCAGCTGGCCC
 CTGCTCGCCGCACTCCTGCAGCTGGCCC
 CTGCTCGCCGCACTCCTGCAGCTGGCCC
 CTGCTCGCCGCACTCCTGCAGCTGGCCC

AGAATCATCACGAAGTGGTGAAGTTCAT
 GGTGTCATGGATAGATGTGTATACTCGC
 GGTGTCATGGATAGATGTGTATACTCGC
 GGTGTCATGGATAGATGTGTATACTCGC
 GGTGTCATGGATAGATGTGTATACTCGC

TTCCAGGAGTACCCCTGATGAGATCGAGT
 GCTCATGGCACCGTGGCCAAAC..AGC
 GCTCATGGCACCGTGGCCAAAC..AGC
 GCTCATGGCACCGTGGCCAAAC..AGC
 GCTCATGGCACCGTGGCCAAAC..AGC

TGACGAGGGCCTGGAGTGTGTGCCCACT
 TGACGATGGCCTGGAGTGTGTGCCCACT
 TGACGATGGCCTGGAGTGTGTGCCCACT
 TGACGATGGCCTGGAGTGTGTGCCCACT
 TGACGATGGCCTGGAGTGTGTGCCCACT

Fig. 5(ii)

| | |
|----------------|--------------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CLASS 56 SUB CLASS |

08/765588
CTA U96/1004

24/52

80

TCCACCATGCCAAGTGGTCCCAGGCTG.
CCGCCCAGGCCCCTGTCTCCCAGCCTGA
CCGCCCAGGCCCCTGTCTCCCAGCCTGA
CCGCCCAGGCCCCTGTCTCCCAGCCTGA
CCGCCCAGGCCCCTGTCTCCCAGCCTGA

160

GGATGTCTATCAGCGCAGCTACTGCCAT
G.....CTACCTGC.CAGCC.CCGGGAG
G.....CTACCTGC.CAGCC.CCGGGAG
G.....CTACCTGC.CAGCC.CCGGGAG
G.....CTACCTGC.CAGCC.CCGGGAG

240

ACATTTCAAGCCATCCTGTGTGCCCT
TGGTGCCCAG.....CTGCGTGACTGT
TGGTGCCCAG.....CTGCGTGACTGT
TGGTGCCCAG.....CTGCGTGACTGT
TGGTGCCCAG.....CTGCGTGACTGT

320

GAGGAGTCCAACATCACCATGCAGATTA
GGGCAGCACCAAGTCCGGATGCAGATCC
GGGCAGCACCAAGTCCGGATGCAGATCC
GGGCAGCACCAAGTCCGGATGCAGATCC
GGGCAGCACCAAGTCCGGATGCAGA...

Fig.5(iii)

25/52

| | |
|-------------|---------------------------|
| | 321 |
| VEGF165 | TGC GGATCAAACCTCACCAAGGCC |
| SOM175 | TCATGATCCGG...TACCCGAGCA |
| SOM175-e6 | TCATGATCCGG...TACCCGAGCA |
| SOM175-e6&7 | TCATGATCCGG...TACCCGAGCA |
| SOM175-e4 | |
| | 401 |
| VEGF165 | AAGAAAAGATAG.....AGCAA |
| SOM175 | AAAAAAGGACAGTGCTGTGAAGCCA |
| SOM175-e6 | AAAAAAGGACAGTGCTGTGAAGCCA |
| SOM175-e6&7 | AAAAAAGGACAGTGCTGTGAAGCCA |
| SOM175-e4 | AAAAAAGGACAGTGCTGTGAAGCCA |
| | 481 |
| VEGF165 |AAGCA..... |
| SOM175 | CTCTGCCCGGGAGCACCCCTCCCC |
| SOM175-e6 | |
| SOM175-e6&7 | |
| SOM175-e4 | CTCTGCCCGGGAGCACCCCTCCCC |
| | 561 |
| VEGF165 | A.....GATCCGCA |
| SOM175 | GCACCACCAGCGCCCTGACCCCCG |
| SOM175-E6 | GCACCACCAGCGCCCTGACCCCCG |
| SOM175-e6&7 | |
| SOM175-e4 | GCACCACCAGCGCCCTGACCCCCG |
| | 641 |
| VEGF165 | TTGAGTTAACGAAACGTACTTGCA |
| SOM175 | TAGAGCTCAACCCAGACACCTGCA |
| SOM175-e6 | TAGAGCTCAACCCAGACACCTGCA |
| SOM175-e6&7 | |
| SOM175-e4 | TAGAGCTCAACCCAGACACCTGCA |

Fig.5(iv)

| | |
|----------------|------------------------------------|
| APPROVED BY | O.G. FIG. CLASS 952700 SURGLASS |
| DRAFTSMAN | |

08/765588
PCT/AU96/00094

26/52

AGCACATAGGAGAGATGAGCTTCCTACA
GTCAGCTGGGGGAGATGTCCCTGGAAAGA
GTCAGCTGGGGGAGATGTCCCTGGAAAGA
GTCAGCTGGGGGAGATGTCCCTGGAAAGA
.....

GACAAGAA....AATCCCTGTGG....
GACAGGGCTGCCACTCCCCACCACCGTC
GATAG.....
GATAG.....
GACAGGGCTGCCACTCCCCACCACCGTC

.....
AGCTGACATCACCCATCCCACTCCAGCC
.....CC
.....
AGCTGACATCACCCATCCCACTCCAGCC

GACGTGTAAATGTTCTGCACAAAC.AC
GACCTGCCGCTGCCGCTGCCGACGCCGC
GACCTGCCGCTGCCGCTGCCGACGCCGC
.....
GACCTGCCGCTGCCGCTGCCGACGCCGC

687

GATGTGACAAGCCGAGGCAGGTGA
GGTGCAGGAAGCTGCGAAGGTGA
GGTGCAGGAAGCTGCGAAGGTGA
. GTGCCGGAAGCTGCGAAGGTGA
GGTGCAGGAAGCTGCGAAGGTGA

Fig.5(v)

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CLASS SUBCLASS GWO 90 2700 |

08/765588
PCT/AU90/00094

27/52

400

GCACAAACAAATGTGAATGCAGACC...A
 ACACAGCCAGTGTGAATGCAGACCTAAA
 ACACAGCCAGTGTGAATGCAGACCTAAA
 ACACAGCCAGTGTGAATGCAGACCTAAA
CCTAAA

480

.....GCCTTGCTCAGAGCGGGAGA
 CCCAGCCCCGTTCTGTTCCGGGCTGGGA

 CCCAGCCCCGTTCTGTTCCGGGCTGGGA

560

.....TTTGTT.....TGTAC..A
 CCAGGGCCCTCTGCCAACGCTGCACCCA
 CCAGGGCCCTCTGCCAACGCTGCACCCA

 CCAGGGCCCTCTGCCAACGCTGCACCCA

640

AGACTCG..CGTTGCAAGGGGAGGCAGC
 AGCTTCCTCCGTTGCCAAGGGCGGGGCT
 AGCTTCCTCCGTTGCCAAGGGCGGGGCT

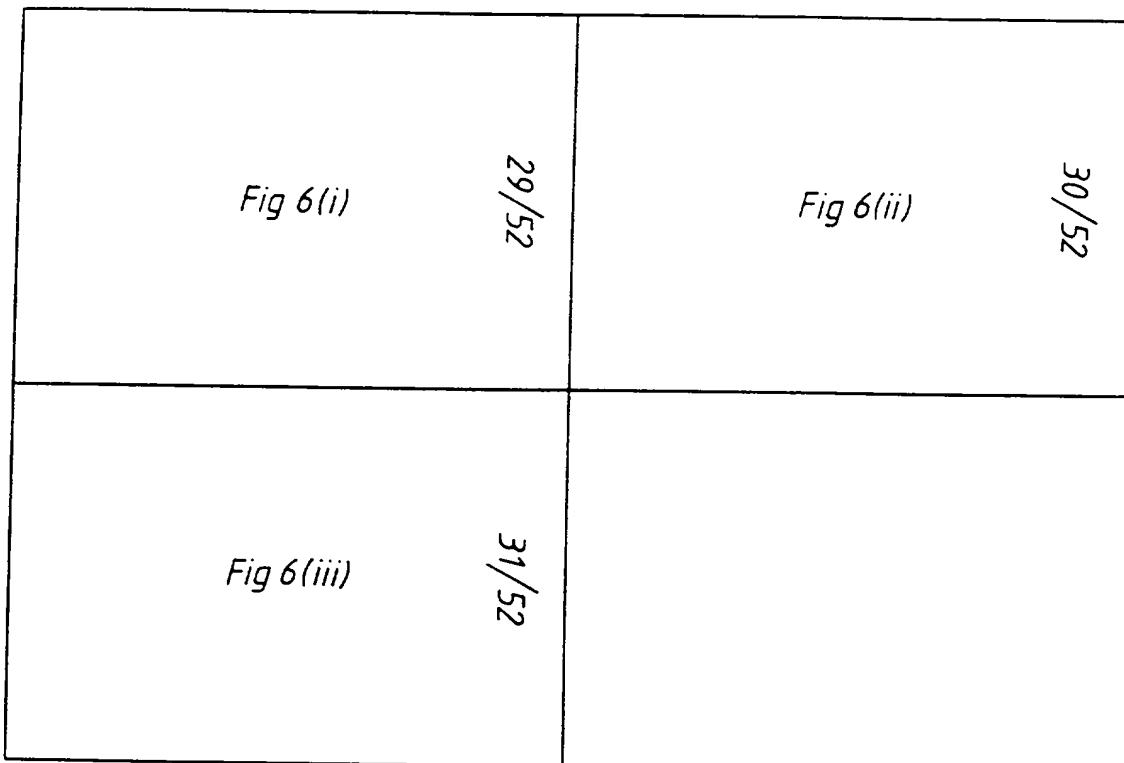
 AGCTTCCTCCGTTGCCAAGGGCGGGGCT

Fig.5(vi)

| | |
|----------------|--------------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CIV 65 SUB 007 ASS |

08/76588
PCT/AU96/00094

28/52



| | |
|----------------|------------------------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CLASS SUBCLASS W096/27007 |

08/765588
PCT/AU96/00094

29/52

| | |
|--|---|
| VEGF ₁₆₅ SOM175 _{Short} | M N F [L L S W V H W S [L A L L Y [L H H A K W S Q A A P M S P [L R R L L A P A Q A P |
| VEGF ₁₆₅ SOM175 _{Short} | I F Q [E Y P D E I E Y I F K P S C V P L M R C G G C C N L T V [E L M G T V A K Q L V P S C V T V Q R C G G C C P |
| VEGF ₁₆₅ SOM175 _{Short} | F L Q [H N K [C E C R P K K D R A L E E H S Q [C E C R P K K K D S A V K P D R A A T P H H |
| VEGF ₁₆₅ SOM175 _{Short} | C K C S C K N T D S R C K A R Q L E L N E R T C R C D K H A A P S T T S A L T P G P A A A A D A A S S V A K |
| OR... | |
| VEGF ₁₆₅ SOM175 _{Long} | M N F [L L S W V H W S [L A L L Y [L H H A K W S Q A A P M S P [L R R L L A P A Q A P |
| VEGF ₁₆₅ SOM175 _{Long} | I F Q [E Y P D E I E Y I F K P S C V P L M R C G G C C N L T V [E L M G T V A K Q L V P S C V T V Q R C G G C C P |
| VEGF ₁₆₅ SOM175 _{Long} | F L Q H N K [C E C R P K K D R A L E E H S Q [C E C R P K K K D S A V K P D R A A T P H H |
| VEGF ₁₆₅ SOM175 _{Long} | G P [C S E R R K H L F V Q [D P Q [T C K C S C K N T D S P R [C T Q H H Q R P D P R [T C R C R C R R R S F L |

Fig.6(ii)

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | SEARCHED CLASS |
| DRAFTSMAN | |

08/765588
PCT/AU96/00094

| | | | | | | | | | | |
|-------------------|-----------|-------------|---------|-----------|-----|-------|-----|-------|-----|----|
| M A E G G G Q N | H H E | V V K F M | D V Y | Q R | S Y | C H | P I | E T L | V D | 60 |
| V S Q P D A P G | H Q R | V V S W I | D V Y | T R | A T | C C | P R | E V V | V V | 55 |
| D E G L E C V P T | E E S N I | T M Q I M R | I K P H | Q G Q | H I | G E M | S | 121 | | |
| D D G L E C V P T | G Q H Q | R M Q I L M | I R . | Y P S S Q | L | G E M | S | 115 | | |

| | | | | | |
|-------------------|-----------------|---------------------|---------------|---------------|-----|
| · · · · · | R Q E N | P C G P C S E R R K | H L F | · V Q D P Q T | 170 |
| R P Q P R S V P G | W D S A P G A P | S P A D I T H | P T P A P G P | S A | 175 |
| P R R | G G A | | | | |

191 30/52
207

| | | | | | | | | | | |
|-------------------|-----------|-------------|---------|-----------|-----|-------|-----|-------|-----|----|
| M A E G G G Q N | H H E | V V K F M | D V Y | Q R | S Y | C H | P I | E T L | V D | 60 |
| V S Q P D A P G | H Q R | V V S W I | D V Y | T R | A T | C Q | P R | E V V | V V | 55 |
| D E G L E C V P T | E E S N I | T M Q I M R | I K P H | Q G Q | H I | G E M | S | 121 | | |
| D D G L E C V P T | G Q H Q | R M Q I L M | I R . | Y P S S Q | L | G E M | S | 115 | | |

| | | | | | | | | | | |
|-------------------------|-------------------|---|---------------|-----------|-----------|-----------|-----------|-----------|-----------|-------|
| R Q E N P | · · · · · | · · · · · | · · · · · | · · · · · | · · · · · | · · · · · | · · · · · | · · · · · | · · · · · | C 170 |
| R P Q P R S V P G | W D S A P G A P | S P A D I T H | P T P A P G P | L | C | 177 | | | | |
| R C K A R Q L E L N E R | T C R C D K P R R | R C Q G R G L E L N P D T C R C R K L R R | | | | | | | | 191 |

222

Fig. 6 (iii)

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SUBCLASS |
| DRAFTSMAN | WO 9627007 |

08/76588
PCT/AU96/00094

31/52

Areas of 100% homology are boxed and conserved residues thought to be involved in homodimerisation are underlined. The VEGF sequence depicted includes the 26 amino acid leader sequence (removal of which gives rise to mature VEGF₁₆₅) giving a total length of 191 amino acids.

Homology of SOM175 to VEGF₁₆₅ is 27% (33%) at the protein level, however within this are blocks of 100% homology. In particular, many structural residues are conserved including those thought to be involved in homodimerisation of VEGF (by comparison with PDGF).
i.e. Cysteine-47
 Proline-70, Cysteine-72, Valine-74
 Arginine-77, Cysteine-78, Glycine-80, Cysteines-81 & 82
 Cysteine-89, Proline-91
 Cysteines 122 & 124

Fig.6(iii)

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SURCLASS |
| DRAFTSMAN | W03 027007 |

08/765588
PCT/AU96/00094

32/52

SPLICE VARIANTS OF SOM175

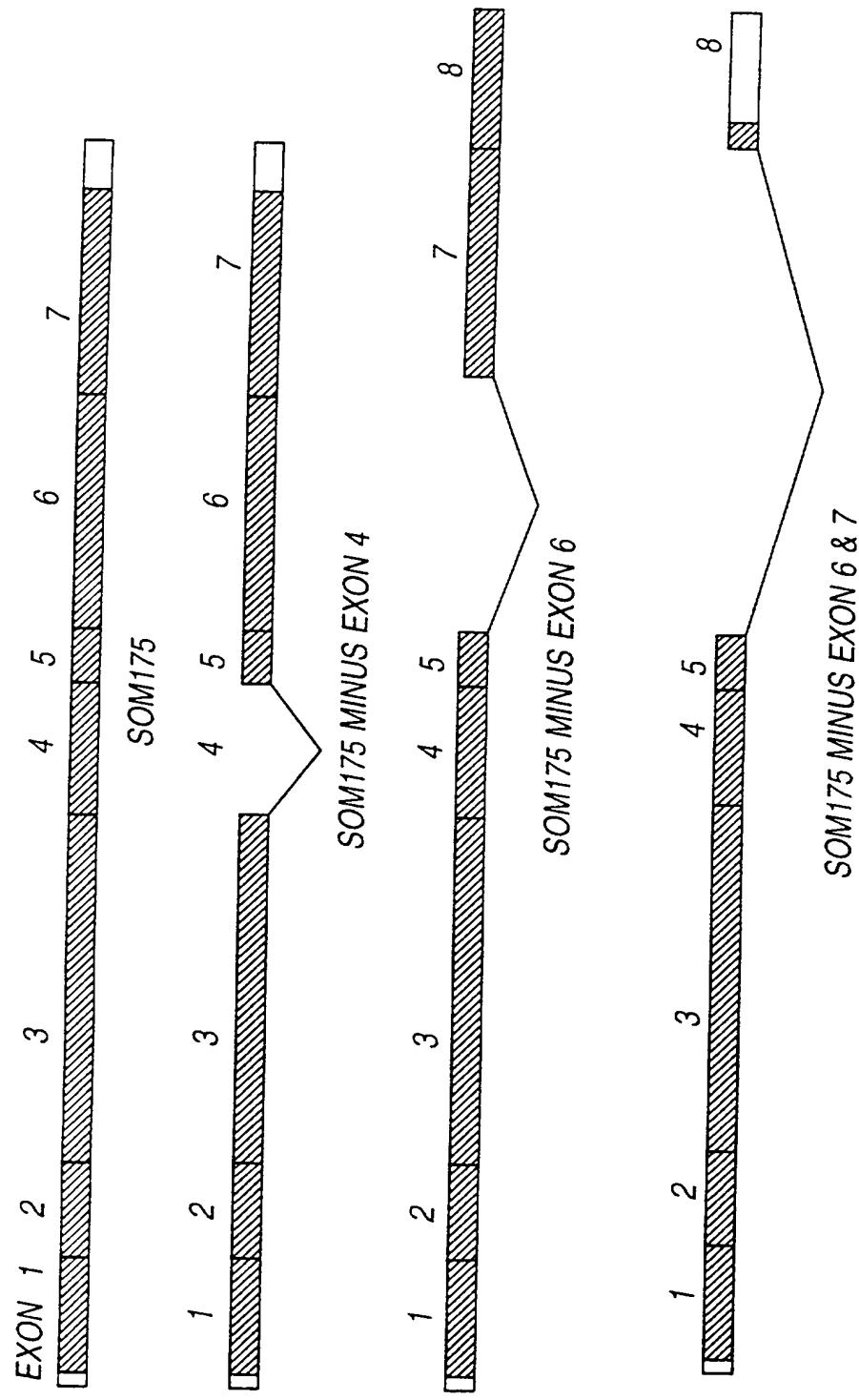


Fig. 7

| | |
|-----------|---------------------|
| APPROVED | O.G. FIG. |
| BY | CLASS 62 SUBCLASS 1 |
| DRAFTSMAN | |

08/765588
PCT/AU96/00094

33/52

GENOMIC STRUCTURE OF HUMAN SOM175

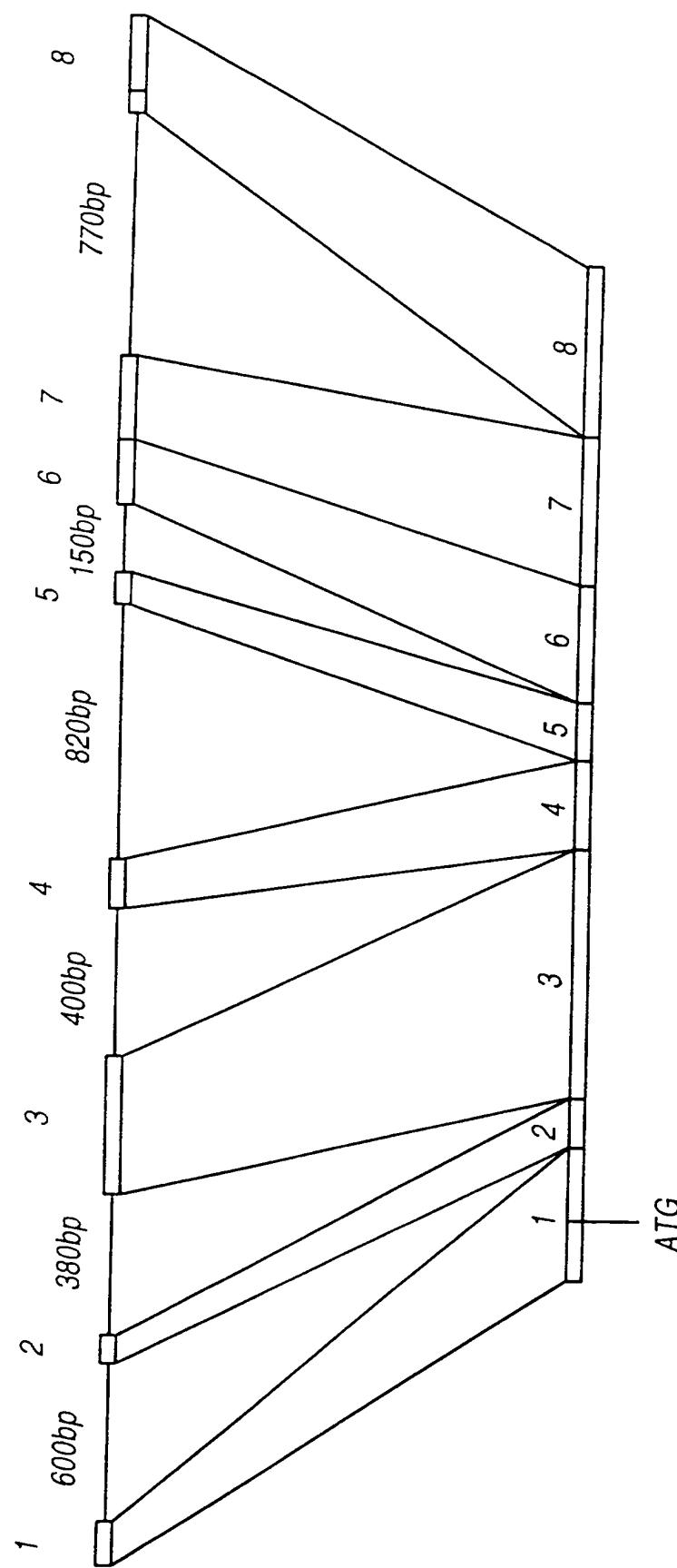


Fig. 8A

| | | |
|----------------|--------------------|----------|
| APPROVED BY | O.G. FIG. CLASS | SUBCLASS |
| DRAFTSMAN | | |

08/765588
PCT/AU96/00094

34/52

| | | | | | |
|--------------|---------------|---------|---------|--------|-------------|
| 5' UTR . . . | ATGAGG | *Exon 1 | (60bp) | GGCCAG | gtacgtgagg |
| tctcccacag | GCCCCCT | Exon 2 | (43bp) | GGAAAG | aataacttaca |
| tctgctccca | TGGTGT | Exon 3 | (187bp) | ATGCAG | gtcccgagatg |
| ctgaatacag | ATCCTC | Exon 4 | (73bp) | ATGCAG | gtgtcaggca |
| actttcaag | ACCTAA | Exon 5 | (34bp) | AGACAG | gtgagtttt |
| ctcctccgta | GGCTGC | Exon 6 | (101bp) | CTCCAG | ccccaggccc |
| cccactccag | CCCCAG | Exon 7 | (109bp) | ACCCAG | acacctgttag |
| ccctgctcag | GTGCCCG | *Exon 8 | (22bp) | AGGTGA | ... 3' UTR |

Fig. 8B

| | |
|-----------|-------------------|
| APPROVED | O.G. FIG. |
| BY | CLASS 16080 CLASS |
| DRAFTSMAN | |

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35/52

| | |
|--------------------|-------------------|
| 36/52 | 37/52 |
| <i>Fig. 9(i)</i> | <i>Fig. 9(ii)</i> |
| 38/52 | 39/52 |
| <i>Fig. 9(iii)</i> | <i>Fig. 9(iv)</i> |

| | |
|----------------|----------------|
| APPROVED BY | O.G. FIG. |
| DRAFTSMAN | CLASS SUBCLASS |

08/765588
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36/52

-163 gcacgagctcaggccgtcgctgcggcgctg
 -103 gggggccgcggaggagccgccccctgcgcc
 -43 ggcggctctggctgaccacccacacccg

 16 CGTCGCCTGCTGCTTGCAC TGCTGCAG
 R R L L L V A L L Q
 ↓
 76 TTTGATGGCCCCAGTCACCAGAAGAAAGTG
 F D G P S H Q K K V

 136 ACATGCCAGCCCAGGGAGGTGGTGGTGCCT
 T C Q P R E V V V P

 196 AAACAACTAGTGCCTCAGCTGTGTGACTGTG
 K Q L V P S C V T V

 256 GGCCTGGAATGTGTGCCCACTGGGAAACAC
 G L E C V P T G Q H

 316 TACCCGAGCAGTCAGCTGGGGAGATGTCC
 Y P S S Q L G E M S

 376 CCTAAAAAAAAGGAGAGTGCTGTGAGGCCA
 P K K K E S A V R P

 436 CAGCCCCGCTCTGTTCCGGGCTGGACTCT
 Q P R S V P G W D S

Fig.9(i)

| | |
|----------------|------------------------|
| APPROVED BY | O.G. FIG. DRAFTSMAN |
| | CLASS SUBCLASS |

08/765588
PCT/AU96/00094

37/52

cgttgcgcgtgcctgcgcggcagggctcgaaa
ccgcggccgggtccccgggtccgcgcacatgg
ccgggctagggcccgATGAGCCCCCTGCTG

M S P L L -17

↓
CTGGCTCGCACCCAGGCCCCCTGTGTCCCAG
L A R T Q A P V S Q 4

GTGCCATGGATAGACGTTATGCACGTGCC
V P W I D V Y A R A 24

CTGAGCATGGAACTCATGGGCAATGTGGTC
L S M E L M G N V V 44

CAGCGCTGTGGTGGCTGCTGCCCTGACGAT
Q R C G G C C P D D 64

↓
CAAGTCCGAATGCAGATCCTCATGATCCAG
Q V R M Q I L M I Q 84

↓
CTGGGAGAACACAGCCAATGTGAATGCAGA
L G E H S Q C E C R 104

↓
GACAGGGTTGCCATACCCACCACCGTCCC
D R V A I P H H R P 124

ACCCCGGGAGCACCCTCCCCAGCTGACATC
T P G A P S P A D I 144

Fig.9(ii)

| | |
|----------------|---------------------------|
| APPROVED BY | O.G.FIG PCT/AU96/00094 |
| DRAFTSMAN | CLASS SUBCLASS |

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PCT/AU96/00094

38/52

496 ATCCATCCCACTCCAGCCCCAGGATCCTCT
 I H P T P A P G S S
 S P R I L

556 CTGACCCCCGGACCTGCCGTTGCCGCTGTA
 L T P G P A V A A V
 P D P R T C R C R C

616 GGGGCCTTAGAGCTCAACCCAGACACCTGTA
 G A *
 R G L E L N P D T C

676 cttccagactccacgggcccggctgcttt
 736 agcacaggcgtaaacctcctcagtctggag
 796 gagctctctgccatctttatctcccaga
 856 atgtctcacctcagggccagggtactctc
 916 ttctggctggctgtctccctcaactatgaa
 976 gggttctgttatgataactgtgacacacac
 1036 gacactaaaaaaaaaaaaaaaaaaaaaaa

Fig.9(iii)

| | |
|----------------|-----------------------------|
| APPROVED BY | O.G. FIG. CLASS SUBCLASS |
| DRAFTSMAN | |

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PC/AU96/00094

39/52

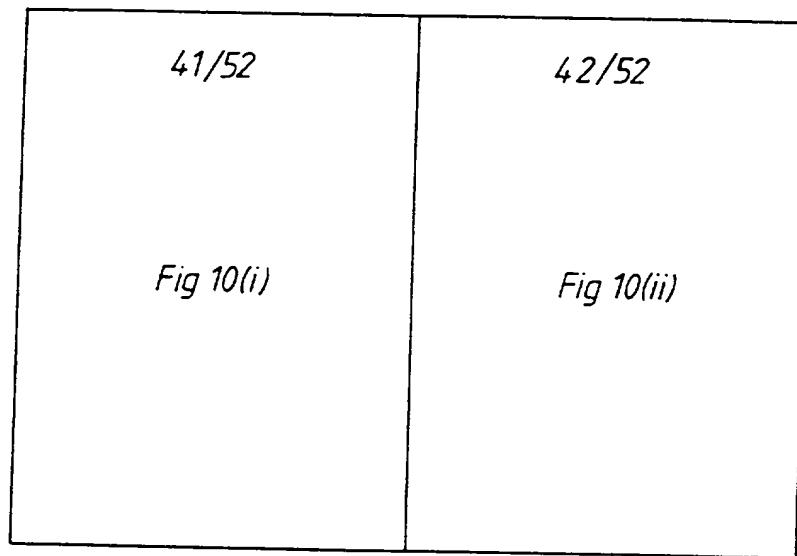
| | |
|---|-----|
| GCCCCGCCTTGCACCCAGCGCCGCCAACGCC | |
| A R L A P S A A N A | 164 |
| C P P C T Q R R Q R | 130 |
| | |
| GACGCCGCCGCTTCCTCCATTGCCAAGGGC | |
| D A A A S S I A K G | 184 |
| R R R R F L H C Q G | 150 |
| | |
| ↓ | |
| GGTGCCGGAAGCCGCGAAAG <u>TGA</u> caagctg | |
| R C R K P R K * | 186 |
| | 167 |
| | |
| tatggccctgtttcacagggagaagagagtgg | |
| gtcactgcccccaggacacctggacccctttaga | |
| gctgccatctaacaattgtcaaggaacctc | |
| tcacttaaccaccctggtcaagtgagcatc | |
| aaccccaaacttctaccaataacgggattt | |
| <u>acacactcacactctgataaaagagatgga</u> | |
| aaaaaaaaaaaaaa | |

Fig.9(iv)

| | |
|----------------|------------------------------|
| APPROVED BY | O.G. FIG. CLASS/ SUBCLASS |
| DRAFTSMAN | |

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PCPAU96/00094

40/52



| | |
|-----------------------------|--|
| APPROVED BY DRAFTSMAN | O.G. FIG. CLASS WO 96/27007 SUBCLASS |
|-----------------------------|--|

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PCT7AU96/0009

41/52

A

hVRF167 -21 MSPLLRRLLAALLQLAPAQAP
||| | | | | | | | | | | | | | | | | | | | | |

mVRF167 -21 MSPLLRRLLLVALQLARTQAP

hVRF167 30 EVVVPLTVELMGTVAKQLVPSC
| | | | : | | | | | | | | | | |

mVRF167 30 EVVVPLSMELMGNVVKQLVPSC

hVRF167 80 ILMIRYPSSQLGEMSLEEHSQL
||||| ||||| : |||||

mVRF167 80 ILMIQYPSSQLGEMSLGEHSQC

hVRF167 130 RPDPTCRCRCCRRSFLRCQGR
| | | | | | | | | | | | : | | | |

mVRF167 130 RPDPTCRCRCKRRFLHCQGR

B

hVRF186 116 RAATPHHRPQPRSVPGWDSAPG
| | | | | | | | | | | | | | | | | | | |

mVRF186 116 RVAIPHHRPQPRSVPGWDSTPG

mVRF186 166 TPGPAVAAVDAAASSIAKGGA*

Fig. 10(i)

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SUBCLASS |
| DRAFTSMAN | |

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42/52

VSQPDAPGHQRKVSWIDVYTRATCQPR 29

||||| : || : || | || || || || || || ||

VSQFDGPGSHQKKVVPWIDVYARATCQPR 29

VTVQRCGGCCPDDGLECVPTGQHQVRMQ 79

||||||| || || || || || || || || || || ||

VTVQRCGGCCPDDGLECVPTGQHQVRMQ 79

ECRPKKKDSAVKPDSPRPLCPRCTQHHQ 129

||||||| : || | : || || | || | || | : :

ECRPKKKESAVRPDSPRILCPPCTQRRQ 129

GLELNPDTCRCKLRR* 167

||||||| || || || | :

GLELNPDTCRCKPRK* 167

APSPADITHPTPAPGPSAHAAPSTTSAL 165

||||||| || || || | || : || | || |

APSPADIIHPTPAPGSSARLAPSAAANAL 165

186

186

Fig.10(ii)

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. CLASS / SUBCLASS |
| DRAFTSMAN | |

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43/52

44/52

45/52

Fig 11(i)

Fig 11(ii)

| | |
|----------------|-----------------------------------|
| APPROVED BY | Q.G. FIG. 100-0000000000000000 |
| DRAFTSMAN | CLASS SUBCLASS |

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44/52

| | | | |
|----------|-----|-----------------------|--|
| mVRF167 | -21 | MSPLLRLRLLVALLQL.. | |
| | | :: : | |
| mVEGF188 | -26 | MNFLLSWVHWTLALLLYLHH | |
| mVRF167 | 25 | TCQPREVVVPPLSMELMGNVV | |
| | | : : : :: : | |
| mVEGF188 | 24 | YCRPIETLVDFQEQYPDEIE | |
| mVRF167 | 75 | QVRMQILMIQYPSSQ.LGEM | |
| | | : : : | |
| mVEGF188 | 74 | NITMQIMRIKPHQSQHIGEM | |
| mVRF167 | 119 |ILCPPC | |
| | | : | |
| mVEGF188 | 124 | QKRKRKKSRFKSWSVHCEPC | |
| mVRF167 | 152 | GLENPDTCRCKPRK | |
| | | : | |
| mVEGF188 | 173 | QLELNERTCRCDKPRR | |

Fig.11(i)

| | |
|-----------------------------|-------------------------------|
| APPROVED BY DRAFTSMAN | O.G. FIG. CLASS / SUBCLASS |
|-----------------------------|-------------------------------|

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45/52

| | |
|----------------------------------|-----|
| AR.TQAPVSQFDGPSHQKKVVPWIDVYARA | 24 |
| : : : : : :: | |
| AKWSQAAPTT.EGEQKSHEVIKFMDVYQRS | 23 |
| KQLVPSCVTVQRCGGCCPDDGLECVPTGQH | 74 |
| : : : :: :: | |
| YIFKPSCVPLMRCAGCCNDEALECVPTSES | 73 |
| SLGEHSQCECRPKKKESAVRPDSPR..... | 118 |
| : | |
| SFLQHSRCECRPKKDRTKPEKKSVRGKGKG | 123 |
| TQRRQR...PDPRTRCRCRCKRFLHCQGR | 151 |
| : : : : : : | |
| SERRKHLFVQDPQTCKCSCKNTDS.RCKAR | 172 |
| | 167 |
| | 188 |

Fig. 11(ii)

| | |
|----------------|----------------|
| APPROVED BY | O.G. FIG. |
| | CLASS SUBCLASS |
| DRAFTSMAN | GW96/2700 |

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PCT/AU96/00094

46/52

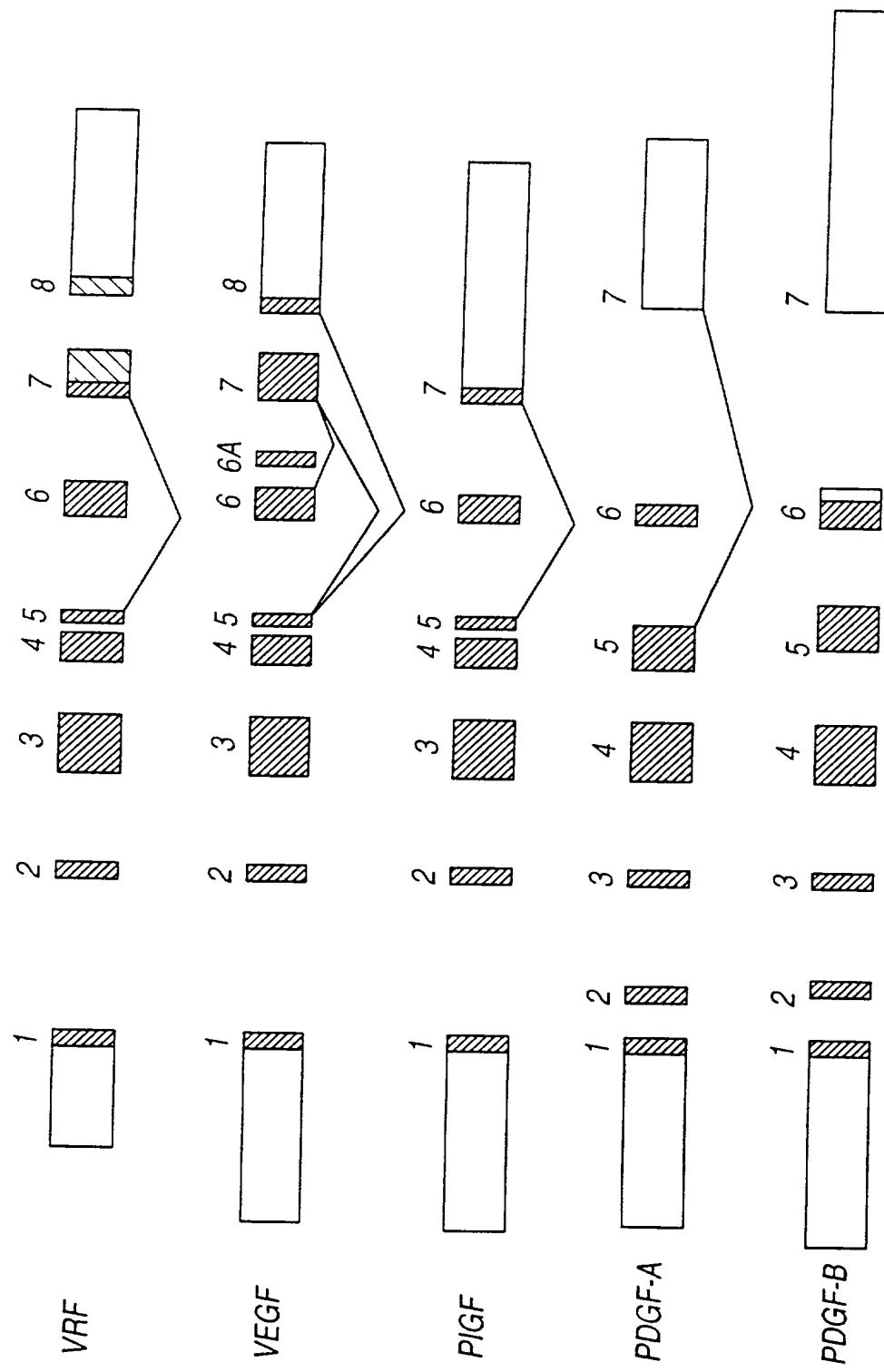


Fig.12

| | | |
|-----------|----------|----------|
| APPROVED | V.G.FIG. | |
| BY | CLASS | SUBCLASS |
| DRAFTSMAN | | |

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PCT/AU96/00094

47/52

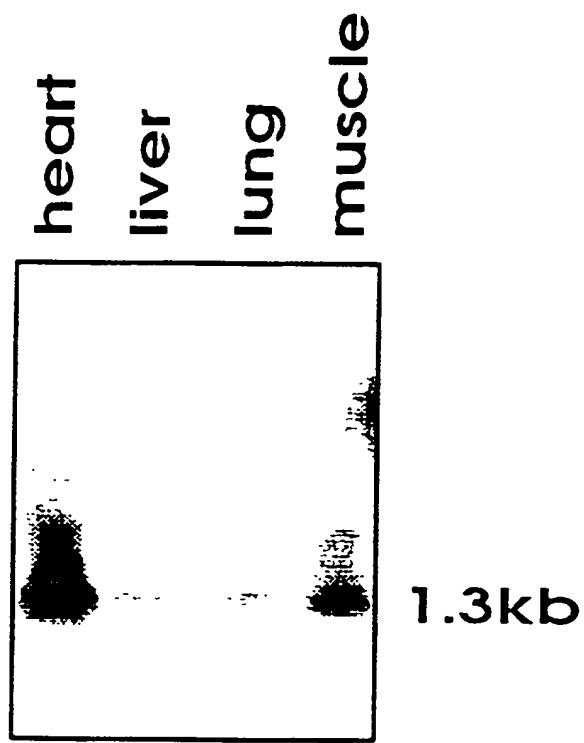


Fig. 13

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SUBCLASS |
| DRAFTSMAN | WO 96/27007 |

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PCT/AU96/00094

49/52

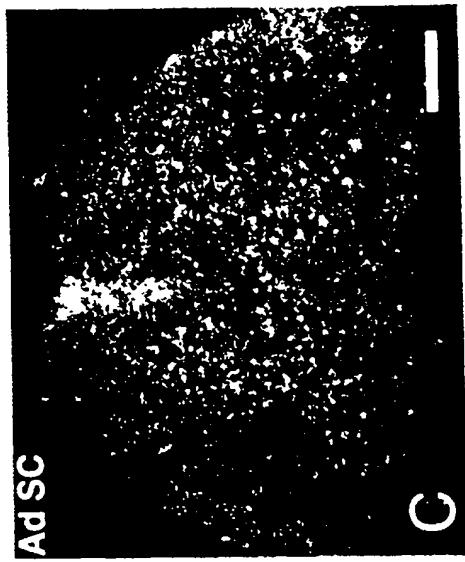
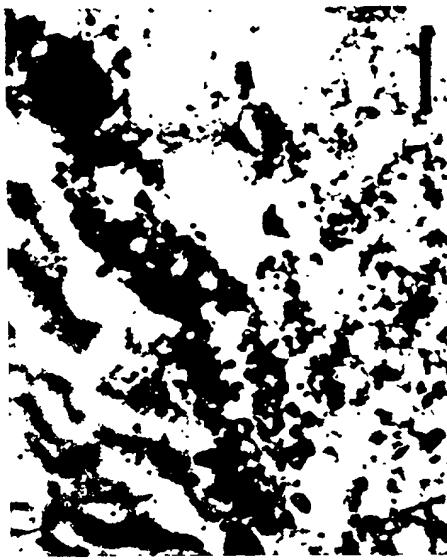


Fig. 15

| | |
|----------------|-------------------------------|
| APPROVED BY | O.G. FIG. CLASS 8280 CLASS |
| DRAFTSMAN | |

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50/52

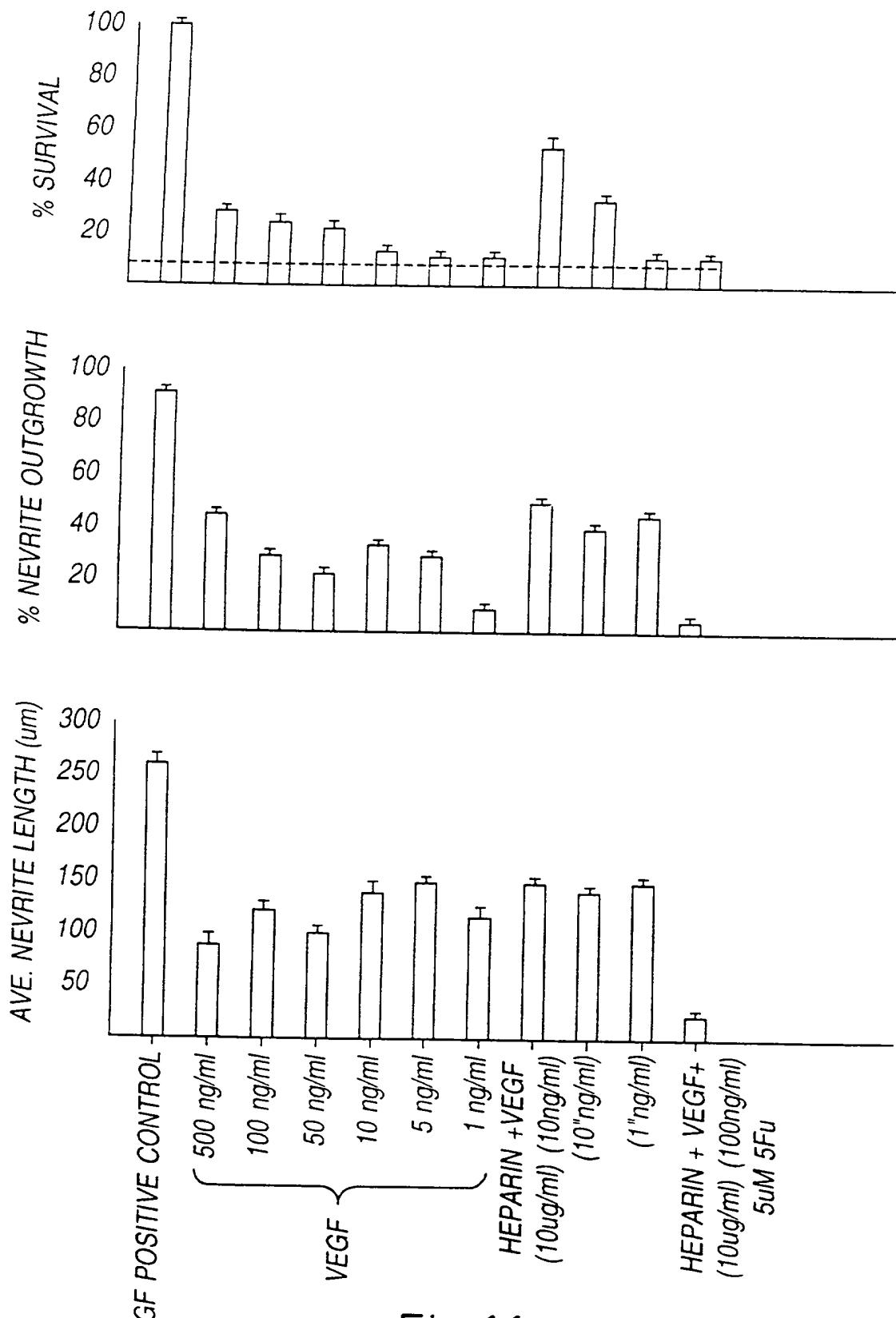


Fig. 16

| | |
|----------------|--------------------|
| APPROVED BY | O.G. FIG. 51/52 |
| DRAFTSMAN | CLASS SUBCLASS |

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PCT/AU96/00094

51/52

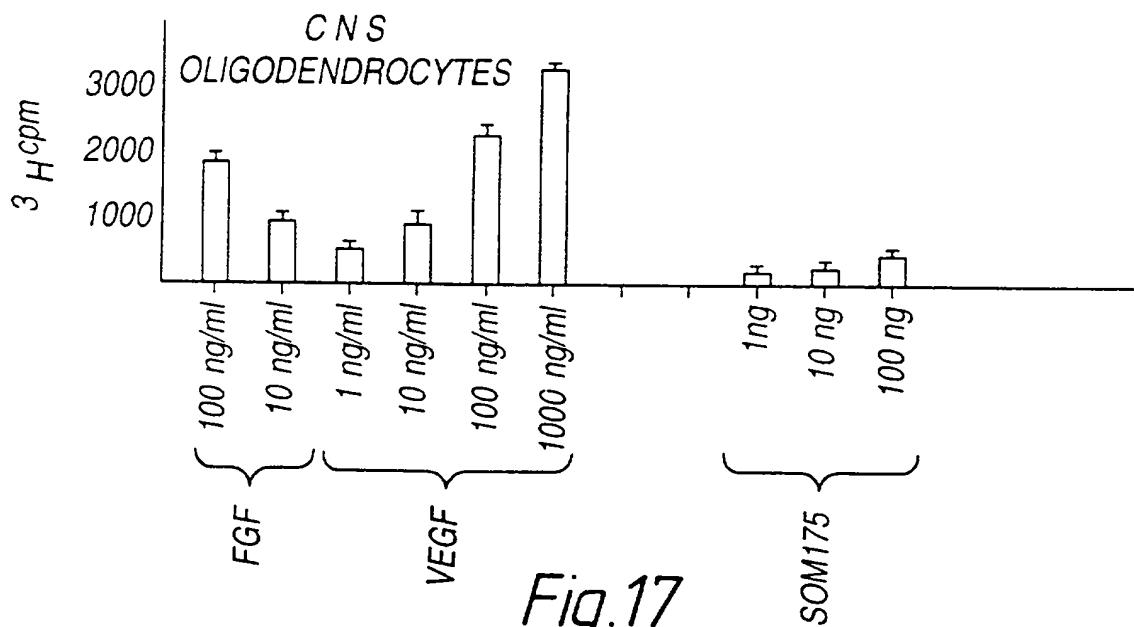
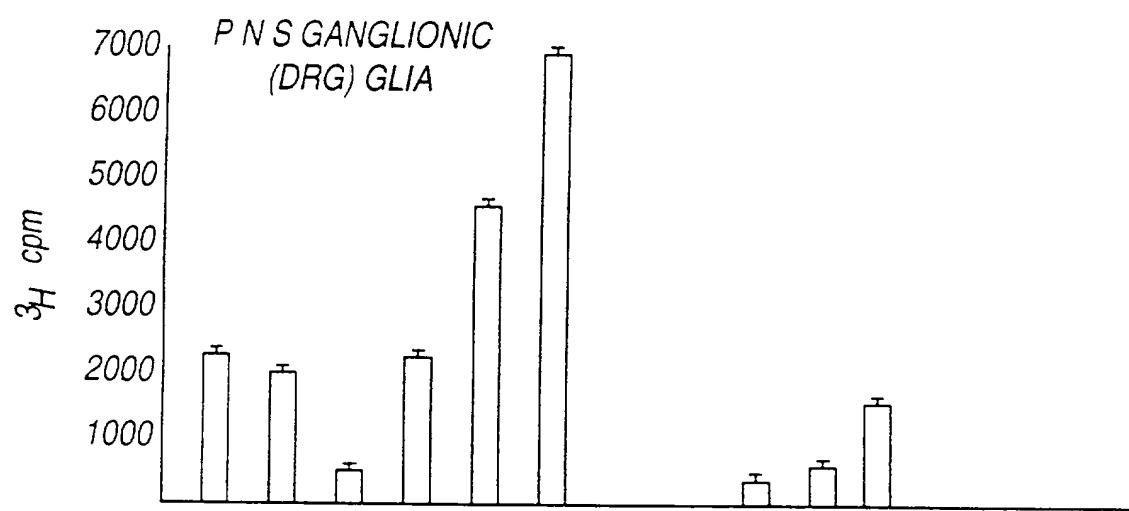
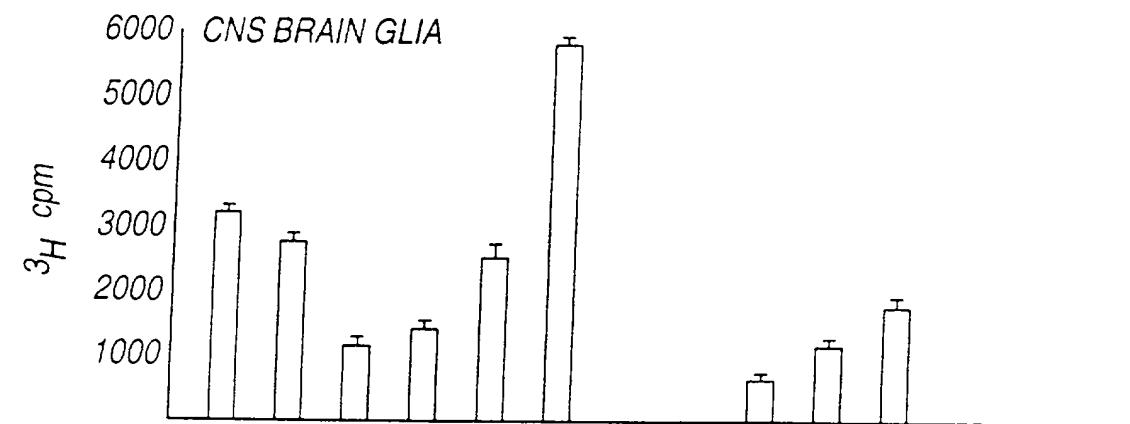


Fig.17
SUBSTITUTE SHEET (RULE 26)

| | |
|-----------|----------------|
| APPROVED | O.G. FIG. |
| BY | CLASS SUBCLASS |
| DRAFTSMAN | WO 96/27007 |

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PCT/AU96/00094

52/52

MOUSE ASTROGLIAL CELLS

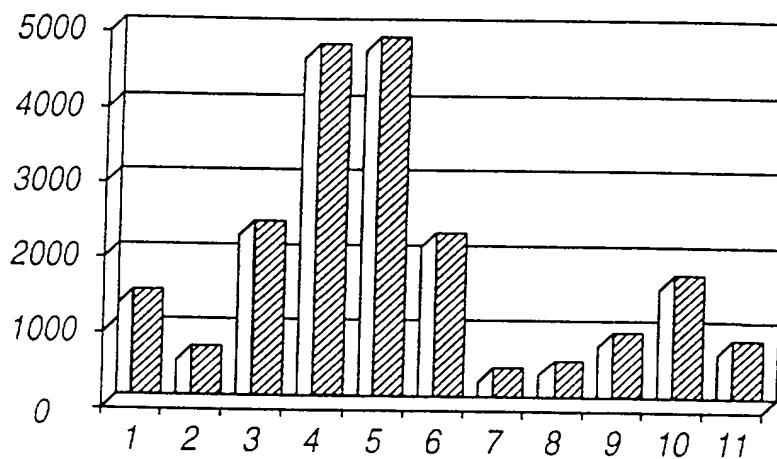


Fig.18

MOUSE OLIGODENDROGLIAL CELLS

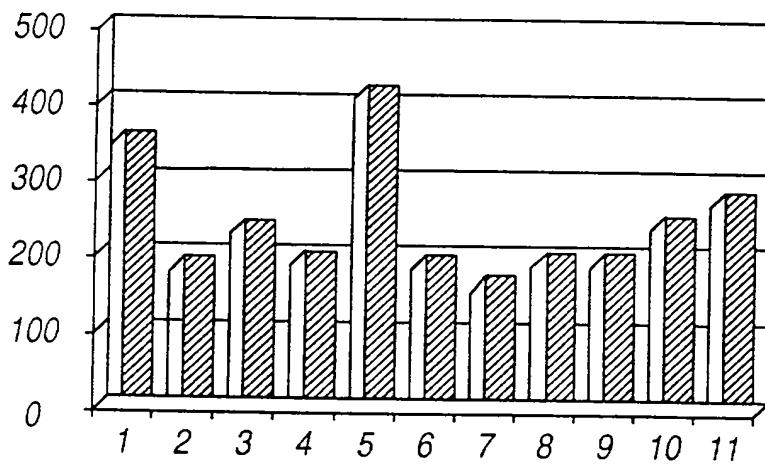


Fig.19

MOUSE FOREBRAIN NEURONS

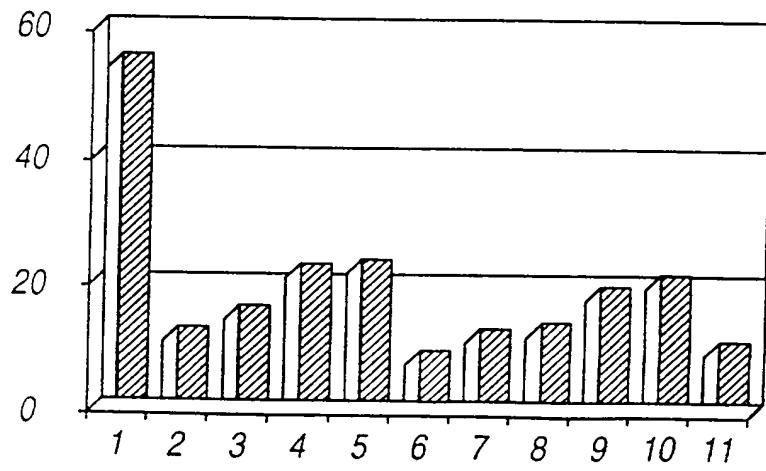


Fig.20

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME"

the specification of which (check only one item below):

 is attached hereto was filed as United States applicationSerial No 08/765,588on 17 December 1996

and was amended

on _____ (if applicable)

 was filed as PCT international applicationNumber PCT/AU96/00094on 22 February 1996

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a)

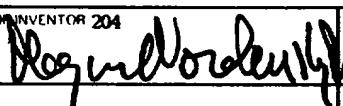
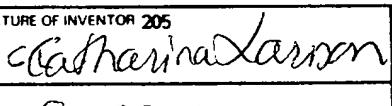
I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

| COUNTRY (If PCT indicate PCT.) | APPLICATION NUMBER | DATE OF FILING (day month year) | PRIORITY CLAIMED UNDER 35 USC 119 |
|-----------------------------------|--------------------|------------------------------------|---|
| Australia | PN 1457/95 | 2 March 1995 | <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO |
| Australia | PN 6647/95 | 20 November 1995 | <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO |
| Australia | PN 7274/95 | 22 December 1995 | <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO |
| | | | <input type="checkbox"/> YES <input type="checkbox"/> NO |
| | | | <input type="checkbox"/> YES <input type="checkbox"/> NO |

| | | | | |
|-----|-------------------------------------|--|---|---|
| 204 | FULL NAME OF INVENTOR <u>204</u> | FAMILY NAME <u>NORDENSKJOLD</u> | FIRST GIVEN NAME <u>Magnus</u> | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY <u>Stockholm</u> | STATE OR FOREIGN COUNTRY <u>Sweden</u> | COUNTRY OF CITIZENSHIP <u>Sweden</u> |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>Koltrastvagen 19</u> | CITY <u>16137 Stockholm</u> | STATE & ZIP CODE/COUNTRY <u>Sweden</u> |
| 205 | FULL NAME OF INVENTOR <u>205</u> | FAMILY NAME <u>LARSSON</u> | FIRST GIVEN NAME <u>Catharina</u> | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY <u>Stockholm</u> | STATE OR FOREIGN COUNTRY <u>Sweden</u> | COUNTRY OF CITIZENSHIP <u>Sweden</u> |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>Dannemoragatan 10m str</u> | CITY <u>11344 Stockholm</u> | STATE & ZIP CODE/COUNTRY <u>Sweden</u> |
| 206 | FULL NAME OF INVENTOR | FAMILY NAME | FIRST GIVEN NAME | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY | STATE OR FOREIGN COUNTRY | COUNTRY OF CITIZENSHIP |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS | CITY | STATE & ZIP CODE/COUNTRY |
| 207 | FULL NAME OF INVENTOR | FAMILY NAME | FIRST GIVEN NAME | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY | STATE OR FOREIGN COUNTRY | COUNTRY OF CITIZENSHIP |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS | CITY | STATE & ZIP CODE/COUNTRY |
| 208 | FULL NAME OF INVENTOR | FAMILY NAME | FIRST GIVEN NAME | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY | STATE OR FOREIGN COUNTRY | COUNTRY OF CITIZENSHIP |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS | CITY | STATE & ZIP CODE/COUNTRY |
| 209 | FULL NAME OF INVENTOR | FAMILY NAME | FIRST GIVEN NAME | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY | STATE OR FOREIGN COUNTRY | COUNTRY OF CITIZENSHIP |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS | CITY | STATE & ZIP CODE/COUNTRY |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

| | | |
|--|---|---------------------------|
| SIGNATURE OF INVENTOR 204  | SIGNATURE OF INVENTOR 205  | SIGNATURE OF INVENTOR 206 |
| DATE <u>9/7/206</u> | DATE <u>9/7/206</u> | DATE |
| SIGNATURE OF INVENTOR 207 | SIGNATURE OF INVENTOR 208 | SIGNATURE OF INVENTOR 209 |
| DATE | DATE | DATE |

Combined Declaration For Patent Application and Power of Attorney (Continued)
(Includes Reference to PCT International Applications)

- ATTORNEY DOCKET NUMBER

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that, those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER
35 U.S.C. 120

| U S APPLICATIONS | | STATUS (Check one) | | |
|--|-----------------|-------------------------------------|---------|-----------|
| U S APPLICATION NUMBER | U S FILING DATE | PATENTED | PENDING | ABANDONED |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| PCT APPLICATIONS DESIGNATING THE U S | | | | |
| PCT APPLICATION NO. | PCT FILING DATE | U S SERIAL NUMBERS ASSIGNED / BY | | |
| | | | | |
| | | | | |
| | | | | |

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. Stephen D. Murphy, Reg. No. 22,002; Leopold Presser, Reg. No. 19,827; William C. Roch, Reg. No. 24,972; Kenneth L. King, Reg. No. 24,223; Frank S. DiGilio, Reg. No. 31,346; Paul J. Esatto, Jr., Reg. No. 30,749; John S. Sensny, Reg. No. 28,757; Mark J. Cohen, Reg. No. 32,211; Richard L. Catania, Reg. No. 32,608 and Donald T. Black, Reg. No. 27,999.

| | | | | |
|--|-------------------------------------|---|--|--|
| Send Correspondence to: | | | | Direct Telephone Calls to (name and telephone number) |
| <u>Scully, Scott, Murphy & Presser</u> <u>400 Garden City Plaza</u> <u>Garden City, NY 11530</u> | | | | <u>Leopold Presser</u> <u>(516) 742-4343</u> |
| 201 | FULL NAME OF INVENTOR <u>101</u> | FAMILY NAME <u>HAYWARD</u> | FIRST GIVEN NAME <u>Nicholas</u> | SECOND GIVEN NAME <u>Kim</u> |
| | RESIDENCE & CITIZENSHIP | CITY <u>Paddington</u> | STATE OR FOREIGN COUNTRY <u>Aux</u> <u>Queensland, Australia</u> | COUNTRY OF CITIZENSHIP <u>Australia</u> |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>13 Prince Street</u> | CITY <u>Paddington, Queensland</u> | STATE & ZIP CODE COUNTRY <u>4064, Australia</u> |
| 202 | FULL NAME OF INVENTOR <u>200</u> | FAMILY NAME <u>WEBER</u> | FIRST GIVEN NAME <u>Gunther</u> | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY <u>Stockholm</u> | STATE OR FOREIGN COUNTRY <u>Sweden</u> <u>SEX</u> | COUNTRY OF CITIZENSHIP <u>Germany</u> |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>Ullriborgsgatan 9nb</u> | CITY <u>11243 Stockholm</u> | STATE & ZIP CODE COUNTRY <u>Sweden</u> |
| 203 | FULL NAME OF INVENTOR <u>300</u> | FAMILY NAME <u>GRIMMOND</u> | FIRST GIVEN NAME <u>Sean</u> | SECOND GIVEN NAME |
| | RESIDENCE & CITIZENSHIP | CITY <u>Taringa</u> | STATE OR FOREIGN COUNTRY <u>Aus</u> <u>Queensland, Australia</u> | COUNTRY OF CITIZENSHIP <u>Australia</u> |
| | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>210 Swann Road</u> | CITY <u>Taringa, Queensland</u> | STATE & ZIP CODE COUNTRY <u>4068, Australia</u> |

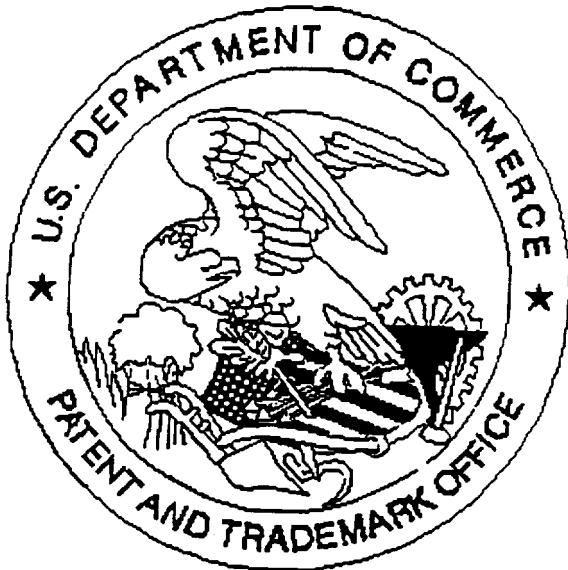
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

| SIGNATURE OF INVENTOR 201 | SIGNATURE OF INVENTOR 202 | SIGNATURE OF INVENTOR 203 |
|---|---|---|
| DATE | DATE | DATE |
|  |  |  |
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